CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the study

Human immunodeficiency virus (HIV) is a member of the genus lentivirus, the family of Retrovirus that contains RNA but lacks DNA, thus it does not replicate outside of living host cells. The entry of HIV into host cells leads to its replication and the establishment of infection, thereby; releasing HIV virons from infected cells which enter the systemic circulation and are carried to widespread sites within the body by antigen presenting cells (APCs) (Fauci *et al*, 1996, Martins and Bandres, 1999). Untreated HIV infection usually progress to Acquired Immune deficiency syndrome (AIDS), a clinical condition that result due to repetitive destruction of the immune system especially the CD4 cells called the T-helper cells which play an important role in immune defense system of the host without much replacement of the cell by the thymus gland. This immune depletion leaves the patients susceptible to various opportunistic infections, malignancies, anaemia and death (Okolie *et al*, 2003). AIDS is confirmed if a person with HIV develops one or more number of severe opportunistic infections or cancer (Sierra *et al*, 2005).

AIDS has been declared a pandemic disease by WHO as it affects countries globally (WH0, 2009). It has affected over 30 million people worldwide (Willey *et al,* 2002; Maplanka, 2007; USAID, 2008). The population affected by HIV in Africa especially sub-Saharan Africa is about 70% (Kumar *et al*, 2006). It is now the leading cause of death in sub-Saharan Africa and the fourth leading cause of mortality worldwide and over 95% of these deaths have occurred among young adults in the developing world (Guatelli *et al*, 2002; USAID, 2008). Highly active antiretroviral therapy (HAART) has generally been taken as the gold standard in the management of HIV patients (Odunukwe *et al*, 2005). With its introduction in 1996, HAART appears to have effectively controlled viral replication in HIV/AIDS patients and has successfully improved their quality of life and prolonged their life-expectancy (De-

larranaga *et al*, 2003; Jacobson *et al*, 2004), with a near normal turnover of CD4 T – cell population (Torti *et al*, 2004; Simon *et al*, 2006).

Anaemia is a clinical condition in which the number of red blood cells and oxygen carrying capacity of blood is reduced. It is usually a sign of an underlying disease and may be mild or severe (Ochei and Kolhatkar, 2000). The aetiology of anaemia in HIV infection often remains unclear. In recent years several attempts have been undertaken to elucidate the mechanisms leading to HIV- associated anaemia (Kreuzer and Rockstroh, 1997). Anaemia in HIV/AIDS has multiple aetiologies, these include: decreased production following suppression of haemopoiesis by lymphoma cells, infection such as tuberculosis and inflammatory cytokines, immune and non-immune haemolysis; or ineffective production due to vitamin B12 or / and folic acid deficiency made worse by therapy with Zidovudine which is known to suppress erythropoiesis (Lim and Levine, 2006). In a study carried out by Olayemi *et al*, (2008), the prevalence of anaemia among HIV patients at the University of Benin Teaching Hospital (UBTH), Benin City, Edo State was found to be 36.74%, hence showing that anaemia was a frequent complication of HIV infection (Sullivan *et al*, 1998).

HIV infection is a systemic disease that has been reported to affect so many organs of the body. It has been implicated in the liver (Houset *et al,* 1990), gonads (Roof and Hall, 2000), bone (WHO, 1994) and adrenal gland (Prasanthai *et al*, 2007).

Patients infected with HIV have an increased risk of developing heart disease. Amongst the most common heart problem associated with HIV are pericarditis (Sudano *et al*, 2006), endocarditis (Miro *et al*, 2003), cancer that affects the heart (Malnick and Goland, 1998), pulmonary hypertension due to inflammation and genetic factors (Pellicelli *et al,* 2001) and coronary artery disease (CAD) (Iloeje *et al*, 2005).The pathogenesis of HIV-associated cardiomyopathy includes direct effects of the human virus on the heart (Malnick and Goland, 1998), the inflammatory response of the host myocardium to the virus (Lewis, 2000) and the presence of autoantibodies (Malnick and Goland, 1998) as well as decreased immunity.

Atherosclerosis is the most frequent cause of morbidity and mortality globally (Ahaneku *et al*, 2001). Some researchers thought that it is caused by a response to the damage to the endothelium from some modifiable factors like high cholesterol, high blood pressure and cigarette smoking (Guldikin *et al*, 2005). Other abnormal lipid levels such as high LDL, high TG and low HDL were risk modifiable factors that were linked with CAD (Ahaneku *et al*, 2001; Kabiri *et al,* 2010). Hyperuricaemia was also linked to CAD (Ahaneku *et al*, 2001; Pichai *et al*, 2009). High levels of triglycerides (TGs) have been linked with diabetes, obesity and low levels of HDL-cholesterol (Kabiri *et al*, 2010).

The proteins associated with lipoproteins transport are called apolipoproteins (apo) and they are divided into families A, B, C and E based on their structure and function. Apo A is mainly involved in HDL particles and has an inverse relationship with CAD. The apo B series is found with LDL particles and found to be an important factor in predicting CAD (Ellas-Scale *et al*, 2007). Some studies have shown that altered apo B concentration is one of the most important risk predictors for cardiovascular disease (Rasouli *et al*, 2006).The ratio of apo A1: apo B may be a useful measure of cardiovascular risk which increases if the ratio is less than 1(Crook, 2006). The apo C series is important in triglyceride metabolism (Aolto *et al*, 1992; Crook, 2006) and apo E is for receptor binding. They are freely interchanged between various lipoproteins (Crook, 2006)·

Before the availability of antiretroviral therapy (ART), studies in HIV infected individuals have shown a variety of lipid abnormalities. Low levels of total cholesterol (TC), High density lipoprotein (HDL) and Low density lipoprotein (LDL) (Grunfeld *et al,* 1992). Early studies suggested that factors such as increased apolipoprotein E levels, increased hepatic synthesis of VLDL and decreased clearance of triglycerides were the contributing factors that cause the lipid abnormalities (Grunfeld *et al,* 1997), while Christeff *et al*, (2002) added that the presence of dyslipidemia in HIV infected individuals may be due to the effects of viral infection, acute-phase reactants, and circulating cytokines.

In 2004, increased levels of TG, Very Low Density Lipoprotein (VLDL), apo E and decreased levels of HDL and apo A1 were observed in HIV group in the US (Malavazi *et al*, 2004). In Uganda, infrequent elevated serum levels in TC, LDL and TG were observed in HIV subjects (Buchaez *et al*, 2008). Elevated serum levels of TG and TC were observed in HIV group in Sagamu, Ogun State, Nigeria (Ogundahunsi *et al*, 2008). Elevated levels of TC and LDL but reduced TG and HDL were observed in South South of Nigeria (Akpan *et al*, 2006).

Also, dyslipidemia has been observed in HIV individuals on ART that could lead to heart diseases. Accelerated atherosclerosis has been seen as side effects of ART. Protease inhibitors (PIs) have been observed in raising LDL (Fontas *et al*, 2004) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) have been also observed in raising HDL-cholesterol (Frerichs *et al*, 2002; Holmberg *et al*, 2002). But some researchers argument continues about whether the causes of these metabolic derangements were as a result of the effect of drugs alone, or from the HIV infection or from the combination of both (Boyle, 2002). Alterations in apo B have been seen in patients receiving combination therapy (with a nucleoside analogue and a protease inhibitor) (Schmitz *et al*, 2001). Also, the levels of lipoprotein particles containing apo C-III and apo E have been reported to have increased in HIV subjects PIs (Bonnet, 2001).

There are cardiac markers available to assess heart diseases such as creatine kinase-MB (CK-MB) which is myocardial specific (Wendy and Robert, 2003). AST is present in higher concentration in cells of cardiac muscle and may be included in the evaluation of heart status (Vasudevan, 2011). Myoglobin is a protein released and elevated during myocardial injury (Crook, 2006). It may also be included in assessing heart function (Kagen *et al*, 1997). Again, total LDH has been found useful in the diagnosis of a myocardial infarction (Crook, 2006). Troponin is also enlisted as a Cardiac marker. Troponin is the most sensitive and specific test for myocardial damage because it has increased specificity compared with creatine kinase (CK-MB) (Mann, 2003).

The incidence of HIV and malaria infections is now a global concern especially in sub Saharan Africa where they are widespread. The co-existence of HIV and malaria infection has been established. Malaria is a life threatening disease that causes major health problems in the tropics and subtropics. It is caused by plasmodium species. Malaria was observed to be endemic in Nigeria and it is the leading cause of morbidity and mortality in the country (Onwujekwe *et al,* 2000). Cohen *et al* (2005), observed an increased prevalence of severe malaria in HIV-infected adults in South Africa. An increased prevalence of severe malaria in HIV-infected adults' subjects in endemic area of Southern Nigeria was observed by Onyenekwe *et al* (2007).

In the South Eastern region of Nigeria, no study to my knowledge has been done to evaluate the effect of HIV/ AIDS with and without malaria infection and with and without Antiretroviral Therapy on the cardiac status and Apolipoprotein profiles of people living with HIV/AIDS. We therefore, hypothesize that apolipoproteins and other markers of cardiac function may exist in the body fluids of subjects living with HIV/ AIDS. Identification of these biomarkers in them will generate data and information that may be useful for better treatment, management and follow-up of individuals.

1.2 Statement of problem

AIDS has been declared a pandemic disease by WHO as it affects countries globally with records of deaths. It is estimated that about 35 million people are living with HIV worldwide, two-third of this infection occurred in sub-Saharan Africa. South Africa has the largest population of HIV subjects globally, followed by Nigeria and India (UNAIDS, 2013). Despite various interventional measures and improved management schemes in recent times, HIV/AIDS still remains a major health problem with a global concern.

HIV infection being a systemic disease has been found to have affected so many organs of the body like the liver (Housset *et al*, 1990), the gonads (Roof and Hall, 2000), bone (WHO, 1994) and the adrenal gland (Pransanthai *et al*, 2007). The HIV infection has also been

implicated in cardiovascular disorders especially in advanced stage of the infection (Cohen *et al*, 2005). Some enzymes have been implicated in HIV infection such as aspartate transferase alkaline transferase, alanine phosphatase (Oluwafemi and Olatunbosun, 2003).

Antiretroviral drugs are used as chemotherapeutic interventions of HIV/AIDS infection, many a times on long term basis. The drugs may present with side effects, most which are not uniquely associated with a particular drug and sometimes may be difficult to identify the cause. HIV itself is capable of producing many of the symptoms that may also occur as drug side effects. Hence it is needful to investigate the effect of HIV and Antiretroviral therapy on Apolipoprotein profile and some other biomarkers of cardiac status in HIV infection.

1.3 Justification of study

Normally, the adipocyte removes lipids from the blood, stores and releases them when they are needed for energy use but report has it that HIV replication alone in human T- cells with or without any influence of antiviral drugs or other factors, can stimulate the production of cellular enzymes and proteins that enhance fatty acids synthesis, increase the quantity of LDL, secrete TGs, alter the lipid transport, metabolism and oxidize lipid (Rasheed *et al* (2008). Stein *et al,* (2012) also reported changes in lipid profiles in HIV infection. Protease inhibitor (PI) had been reported to cause myocardial infarction, dyslipidaemia and lipohypertrophy in HIV subjects with long term exposure to HAART (Friis-Moller *et al*, 2003) and lipoatrophy with thymidine analogues (Nolan and Mallal, 2005).

There has been report that individuals infected with HIV have increased risk of developing cardiopathy (Sudano *et al*, 2006). Hence, cardiac biomarkers will be measured to evaluate heart function especially in HIV infection, since they are released in blood during heart damage (Vasadevan *et al*, 2011). But not much had been documented on the pattern of cardiac markers such as Troponins, Myoglobin and Apolipoproteins in HIV infection on Lamivudine, Nevirapine and Zidovudine, in the South Eastern region of Nigeria, hence their evaluation in this study. Identification of these biomarkers in HIV/AIDS subjects may inform

the need for routine investigation and early and better treatment of cardiac enzymopathies and organ abnormalities in the management and follow-up of HIV/AIDS subjects.

1.4 Aim of the study

The aim of this study is to evaluate the levels of apolipoprotein profile and other markers of cardiac function in HIV/AIDS subjects on Anti retroviral therapy in NAUTH Nnewi, South East, Nigeria.

1.5 Specific objectives of the study

The specific objectives of this study are:

- To determine the serum level of Apolipoprotein profile (Apo A_1, A_2, B, C_2, C_3 and E) in participants presenting with HIV infection, in those on ART and Control as an index for cardiac status.
- To evaluate the serum levels of Troponin, Myoglobin, CK-total, CK-MB, AST and LDH in HIV infected subjects and in subjects on ART.
- To determine the serum level of lipid profile (total Cholesterol, HDL, LDL and Triglyceride) in HIV infected subjects and in subjects on ART.
- To evaluate the blood CD4 + T cell counts in HIV infected subjects and in subjects on ART.
- To determine if there will be an adverse or an improved effect of Anti retroviral therapy on the Apolipoprotein profile, CD4 count and other markers of cardiac function in HIV infected subjects on ART.
- To determine if there will be any effect of malaria endemicity on the Apolipoproteins, lipid profile and other markers of cardiac function in HIV infected subjects and in subjects on ART.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Review of HIV/AIDS

2.1.1 History of HIV/AIDS

AIDS was first clinically observed in 1981 in the United States (Gerald *et al*, 2010). The initial cases were a cluster of injection drug users and gay men (men that have sex with men) with no known cause of impaired immunity who showed symptoms of *Pneumocystis carinii pneumonia* (PCP), a rare opportunistic infection that was known to occur in people with much compromised immune system (Gottlieb, 2006). Soon thereafter, additional gay developed a previously rare skin cancer called Kaposi's sarcoma (KS) (Friedman-Kien, 1981; Hymes *et al*, 1981). Many more cases of PCP and KS emerged, alerting U.S Centers for Disease Control and Prevention (CDC) and a CDC task force was formed to monitor the outbreak (Basavapathruni and Anderson, 2007).

In the beginning, the CDC did not have an official name for the disease, often referring to it by way of the diseases that were associated with it, for example, lymphadenopathy, after which the discoverers of HIV originally named the virus (CDC, 1982; Barre-Sinoussi *et al,* 1993). In the general press, the term GRID, which stood for Gay- related Immunodeficiency had been coined (Altman, 1982). The CDC, in search of a name and looking at the infected communities realized that the term GRID was misleading and AIDS was introduced at a meeting in July 1982 (CDC, 1982), by September 1982, the CDC started using the name AIDS.

In 1983, two separate research groups led by Robert Gallo and Luc Montagnier independently declared that a novel retrovirus may have been infecting AIDS patients and published their findings in the same issue of the journal Science (Gallo *et al,* 1983; Barre-Sinoussi, 1983). Gallo claimed that a virus his group had isolated from an AIDS patient was strikingly similar in shape to other human T- lymphotropic viruses (HTLVs) his group had been the first to isolate. Gallo's group called their newly isolated virus HTLV-111. At the

same time, Montagnier's group isolated virus from a patient presenting with swelling of the lymph node of the neck and physical weakness, two classic symptoms of AIDS. Contradicting the report from Gallo's group, Montagnier and his colleagues showed that core proteins of this virus were immunologically different from those of of HTLV-1. Montagnier's group named their isolated virus lymphadenopathy- associated virus (LAV). As these two viruses turned out to be the same, in 1986, LAV and HTLV-111 were renamed HIV (Wotherspoon, 2001).

Both HIV-1 and HIV-2 are believed to have originated in non- human primates in West – central Africa and to have transferred to humans (a process known as zoonosis) in the early 20th century (Sharp and Hahn, 2011). HIV -1 appears to have originated in Southern Cameroon through the evolution of SIV, a Simian Immunodeficiency Virus (SIV) that infects wild chimpanzees (Gao *et al*, 1999; Keele *at al*, 2006). HIV-1 is thought to have jumped the species barrier on at least three separate occasions, giving rise to the three groups of the virus, M, N and O (Sharp *et al*, 2001).

There is evidence that hunters or bush meat vendors commonly acquire SIV (Kalish, 2005). However, SIV is a weak virus and it is typically suppressed by the human immune system within weeks of infection. It is thought that several transmissions of the virus from individual to individual in quick succession are necessary to allow it enough time to mutate into HIV (Marx *et al*, 2001). Furthermore, due to its relatively low person- to- person transmission rate, it can only spread throughout the population in the presence of one or more of high – risk transmission channels, which are thought to have been absent in Africa prior to the 20th century.

Figure 2.1: Picture of monkeys that are sources of SIV (Adapted from Kalish *et al*, 2005).
Left to right: the African green monkey source of SIV, the sooty mangabey source of HIV-2 and the chimpanzee source of HIV-1.

2.1.2 HIV structure and genome

HIV is different in structure from other *retroviruses*. It is roughly spherical (McGovern *et al*, 2002) with a diameter of about 120nm, around 60 times smaller than a red blood cell, yet large for a virus (Fisher *et al*, 2007). It is composed of two copies of positive single- stranded RNA that codes for the virus's nine genes enclosed by a conical capsid made up 2,000 copies of the viral protein p24. The single-stranded RNA is tightly bound to nucleocapsid protein p 6, p7 and enzymes needed for the development of the viron such as reverse transcriptase, protease, ribonuclease and integrase. The p6 and p7 associate with genomic RNA to protect the RNA from digestion from nucleases. The reverse transcriptase transcribed the viral RNA into double DNA, while the integrase integrates the DNA produced by reverse transcriptase into the host' genome. A matrix composed of viral protein p17 surrounds the capside ensuring the integrity of the virion particle. This is in turn surrounded by the viral envelope that is composed of two layers of fatty molecules called phospholipids taken from the membrane of a human cell when a newly formed virus particle buds from the cell. Embedded in the viral envelope are proteins from the host cell and about 70 copies of a complex HIV protein that protrudes through the surface of the virus molecule called glycoprotein (gp) 120 and a stem consisting of three gp 41 molecules that anchor the structure into the viral envelope. This glycoprotein complex enables the virus to attach to and fuse with target cells to initiate the infectious cycle. Both these surface proteins, especially gp 120, have been considered as targets for future treatments or vaccines against HIV (Chan *et al*, 1997; Ferguson *et al*, 2002; Gomez & Hope, 2005; Sriram, 2006; Zhu *et al*, 2008). The RNA genome consists of at least seven structural landmarks (LTR, TAR, RRE, PE, SLIP, CRS and INS) and nine genes (*gag, pol, env, tat, rev, nef, vif, vpr, vpu*) and sometimes a tenth *tev,* which is a fusion of *tat* and *rev*, encoding 19 proteins. Three of these major genes, *gag, pol* and *env*, contain information needed to make the structural proteins for new virus particles. For example, *env* codes for a protein called gp 160, the protein that enable the virus to attach to and fuse with the target cells, that is broken down by a cellular protease to form gp 120 and

gp 41; *pol* codes for viral enzymes reverse transcriptase, integrase and HIV protease while *gag* a group specific antigen code for matrix protein, p17. The six remaining genes, *tat, nef, vif, vpr* and *vpu* in the case of HIV-2 are regulatory genes for proteins that control the ability of HIV to infect cells, replicate new copies of virus or cause disease. (Chan *et al*, 1997; Levy, 2006). When a western blot test is used to detect HIV infection, p24 is one of the three major proteins tested for, along with gp120, gp160 and gp41 (Sriram, 2006; Zhu *et al*, 2008).

Fig 2.2: Diagram of HIV virus (Adapted from Fisher *et al*, 2007).

2.1.3 Tropism

The term tropism refers to cell types which HIV infects. HIV can infect a variety of immune cells such as CD^+ T cells, macrophages and microglial cell. HIV-1 entry to macrophages and CD^+ T cells mediated through interaction of the viron envelope glycoproteins (gp 120) with the CD4 molecule on the target cells and also with chemokine co-receptors (Chan *et al*, 1997). Macrophage (M- tropic) strains of HIV-1 or non-syncytia- inducing strains (NSI) use the β- chemokine receptor CCR5 for entry and are , thus, able to replicate in macrophages and CD^+ T cells (Coakley *et al*, 2005). This CCR5 co- receptor is used by almost all primary HIV-1 isolates regardless of viral genetic subtype. Indeed, macrophages play a key role in several critical aspects of HIV infection. They appear to be the first cells infected by HIV and perhaps the source of HIV production when CD4 cells become depleted in the patient. Macrophages and microglial cells are the cells infected by HIV in the central nervous system, thereby; their populations are depleted when infected by HIV. In tonsils and adenoid of HIV-infected patients, macrophages fuse into multinucleated giant cells that produce huge amount of virus. Some people are resistant to certain strains of HIV (Murdoch & Finn, 2000; Tang and Kaslow, 2003). For example, people with the CCR5-32 mutation are resistant to infection with R5 virus, as the mutation stops HIV from binding to this co-receptor, reducing its ability to infect target cells.

Sexual intercourse is the major mode of HIV transmission. Both X4 and R5 HIV are present in the seminal fluid, which is passed from a male to his sexual partner. The virons can then infect numerous cellular targets and disseminate into the whole organism. However, a selection process leads to a predominant transmission of the R5 virus through this pathway (Zhu *et al*, 1996). However, a selection process is still under investigation, but one model is that spermatozoa may selectively carry R5 HIV as they possess both CCR3 and CCR5 but not CXR4 on their surface (Muciaccia *et al,* 2005) and that genital epithelial cells preferentially sequester X4 virus (Berlier *et al*, 2005). In patients infected with subtype B HIV-1, there is often a co-receptor switch in late stage disease and T –tropic variants appear

that can infect a variety of T cells through CXCR4 (Clevestig *et al*, 2005). These variants then replicate more aggressively with heightened virulence that causes rapid T cell depletion, immune system collapse and opportunistic infections that mark the advent of AIDS (Moore, 1997; Murdoch & Finn, 2000). Thus, during the course of infection, viral adaptation to the use of CXCR4 instead of CCR5 may be a key step in the progression to AIDS.

HIV-2 is much less pathogenic than HIV-1 and is restricted in its worldwide distribution. The adoption of 'accessory genes' by HIV- 2 and its more promiscuous pattern of co-receptor usage may assist the virus in its adaptation to avoid innate restriction factors present in host cells. Adaptation to use normal cellular machinery to enable transmission and productive infection has also aided the establishment of HIV-2 replication in humans. (Cheney and McKnight, 2010).

Fig.2.3: Diagram of the immature and mature forms of HIV (Adapted from Fisher *et al*, 2007)

2.1.4 Epidemiology

In 2008 in the United States approximately 1.2 million people were living with HIV, resulting in about 17,500 deaths, of which 20% of infected Americans were unaware of their infection (CDC, 2011).

In 2010, over 33 million people have HIV infection worldwide. Of these approximately 16.8 million are women and 3.4 million are less than 15 years old. It resulted in about 1.8 million deaths in 2010(UNAIDS Outlook, 2010; UNAIDS 2011), down from a peak of 2.2 million in 2007 (UNAIDS, 2007). In 2010, two- third of about 34 million people with the infection occurred in sub-Saharan Africa. South Africa has the largest population of HIV patients globally, followed by Asia, India and Nigeria (UNAIDS Outlook, 2010).

In 2012, the number of positive people in Africa receiving anti-retroviral treatment was over seven times the number receiving treatment in 2005, with nearly I million added in the last year alone (WHO, 2006). The number of AIDS-related deaths in sub-Saharan Africa in 2011 was 33 percent less than the number in 2005. The number of new HIV infections in sub-Saharan Africa in 2011 was 25 percent less than the number in 2001 (WHO, 2006).

In Nigeria, the HIV prevalence rate among adults ages 15 – 49 is 3.8 percent. Nigeria has the second largest number of people living with HIV. The prevalence rate of HIV in Nigeria has declined to 3.1 percent (NACA, 2011). The HIV epidemic in Nigeria is complex and varies widely by region. In some states, the epidemic is more concentrated and driven by high-risk behaviors, while other states have more generalized epidemics that are sustained primary by multiple sexual partnerships in the general population. Youth and young adults in Nigeria are particularly vulnerable to HIV, with young women at higher risk than young men. There are many risk factors that contribute to the spread of HIV, including prostitution, high- risk practices among itinerant workers, high prevalence of sexually Transmitted infection (STI), clandestine high-risk heterosexual and homosexual practices, international trafficking of women and irregular blood screening (United States Department of State, 2008).

2.1.5 Pathophysiology of AIDS

HIV being a Lentivirus is transmitted as single stranded enveloped RNA virus and upon entry into the host, its viral RNA genome is converted into double stranded proviral DNA by the reverse transcriptase of the HIV. The proviral DNA is then inserted into the host cell genomic DNA by the integrase enzyme of the HIV. Once the virus infects the cell, two pathways are possible, either the virus becomes dormant and the infected cell continues to function or the virus becomes active and replicates within cells. The Infected cells then release virus by surface budding or they undergo lysis with the release of new HIV virus, which can then infect additional cells. Also, infected cells undergo the process of apoptosis (programmed cell death) and it is increased with progression of HIV disease but it is diminished with effective antiretroviral therapy (Gougeon, 2003; Vandegraaff & Engelman, 2007).

HIV crosses the epithelia cell of the host by a process of transcytosis fusion or by endocytosis. After entering the body, the viral particle infects human cells through CD4 cell-surface receptor molecules (Ferguson *et al*, 2002) but it may infect those that lack CD4 receptors such as fibroblasts, monocytes, tissue macrophages, natural killer (NK), dendritic cells, hematopoietic stromal cells, and microglial cells in brain through the use of complement receptor site for entry. Cells within the brain and bowel that possess receptors also serve as point of entry of the virus into the host (Stebbing *et al*, 2004; Sierra *et al*, 2005).

After the virus enters the body, there is a period of rapid viral replication, leading to an abundance of virus in the peripheral blood. During primary infection, the level of HIV may reach several million viruses particles per millilitre of blood (Piatak *et al*, 1993). This response is accompanied by a marked drop in the number of circulating $CD4^{+}$Tcells. The acute viraemia is almost invariable associated with activation of $CD8^{+}$Tcells, which kill HIV-infected cells and subsequently with antibody production or seroconversion. The $CD8^{+}$Tcell response is thought to be important in controlling virus levels, which peak and then decline, as the $CD4^{+}$Tcell counts recover. A good $CD8^{+}$Tcell response has been linked to slower

disease progression and a better prognosis, though it does not eliminate the virus (Pantaleo et al, 1997).

The pathophysiology of AIDS is complex (Guss, 1994). Ultimately, HIV causes AIDS by depleting $CD4^{+}$Tcells. This weakens the immune system and allows opportunistic infections. T cells are essential to the immune response and without them the body cannot fight infections or kill cancerous cells. The mechanism of $CD4^{+}$Tcell depletion differs in the acute and chronic phases (Hel *et al*, 2006). During the acute phase, HIV- induced cell lysis and killing of infected cells by cytotoxic T cells accounts for $CD4^{+}$Tcell depletion, although apoptosis may also be a factor. During the chronic phase, the consequences of generalized immune activation coupled with the gradual loss of the ability of the immune system to generate new T cells appear to account for the slow decline in $CD4^{+}$Tcell numbers (Zuckerman, 2007). Continuous HIV replication causes a state of generalized immune activation persisting throughout the chronic phase (Appay and Sauce, 2008).

Although, the symptoms of immune deficiency characteristic of AIDS do not appear for years after a person is infected, the bulk of $CD4^{+}$Tcell loss occurs during the first weeks of infection, especially, especially in the intestinal mucosa, which harbors the majority of the lymphocytes found in the body (Mehandru *et al*, 2004).

2.1.6 HIV disease staging and classification

(a) WHO clinical staging (2006)

This was developed by World Health Organisation (WHO) in 1990 and revised in 2006. The staging is based on clinical findings that guide the diagnosis, evaluation and management of HIV/AIDS and it does not require a CD4 cell count. This staging system is used in many countries to determine eligibility for antiretroviral therapy, particularly in setting in which CD4 test is not available. Clinical stages are categorized as 1 through 4, progressing from primary HIV infection to advanced HIV /AIDS. These stages are defined by specific clinical conditions or symptoms.

Primary HIV infection.

This stage of infection also known as acute retroviral syndrome occurs when HIV first infects the cell; it lasts for a few weeks and is often accompanied by a short flu-like illness. During this stage there is a large amount of HIV in the peripheral blood and the immune system begins to respond to the virus by producing HIV antibodies and cytotoxic lymphocytes. The antibody testing is positive at this stage when HIV infection exceeds two weeks.

Clinical HIV stage 1 (asymptomatic)

This stage lasts for some years and, as its name suggests, is free from major symptoms, although there may be swollen glands. There is generalized lymphadenopathy.

Clinical HIV stage 2 (symptomatic)

This is the stage of symptomatic phase. The level of HIV in the peripheral blood drops to very low levels but people remain infectious. This stage is accompanied by a marked drop in the numbers of circulating $CD4^+$ T cells. At this point the body immune system has been compromised and severely damaged by HIV. This is so because the lymph nodes and tissues have become damaged or 'burnt out' because of the years of activity; HIV mutates and becomes more pathogenic, and the body fails to keep up with replacing the T helper cells that are lost.

So, as the immune system fails, symptoms develop. Initially many of the symptoms are mild, but as the immune system deteriorates the symptoms worsen. Symptomatic HIV infection is mainly caused by the emergence of opportunistic infections and cancers that the immune system would normally have prevented. This stage of HIV infection is often characterized by unexplained moderate weight loss, recurrent respiratory infections (sinusitis, tonsillitis, otitis media, pharyngitis), Herpes zoster, dermatitis, oral candidiasis, fungal nail infections.

Clinical HIV stage 3

This stage of HIV infection is often characterized by multi-system disease and infections such as unexplained chronic diarrhea for longer than one month, unexplained persistent fever for longer than one month, unexplained severe weight loss, persistent oral candidiasis

(thrush), bacterial infections, pulmonary tuberculosis, oral hairy leukoplakia, acute necrotizing ulcerative gingivitis, stomatitis or periodontitis, unexplained anaemia, neutropenia, chronic thrombocytopenia.

Clinical HIV stage 4 (progression to AIDS)

As the immune system becomes more and more damaged, with low level of CD4 cell counts, the illnesses that occur become more and more severe leading eventually to AIDS. HIV individuals in this class develop one or more of a specific number of severe opportunistic infections or cancers such as HIV wasting syndrome, pneumocystis pneumonia, recurrent severe bacterial pneumonia, chronic herpes simplex infection (orolabial, genital or anorectal site for longer than one month) or visceral herpes at any other site, oesophageal candidiasis, extrapulmonary tuberculosis, kaposi's sarcoma, central nervous system toxoplasmosis, HIV encephalopathy, cryptococcosis, mycobacteria infection, progressive multifocal leukoencephalopathy, chronic cryptosporidiosis, chronic isosporiasis, disseminated mycosis, salmonella bacteremia, lymphoma, invasive cervical carcinoma and candida of the trachea, bronchi or lungs.

(b) CDC (1992) classification system

Center for Disease Control and prevention (CDC) disease staging assess the severity of HIV disease by CD4 cell counts and by the presence of specific HIV related conditions. Clinical stages are categorized as A, B and C progressing from asymptomatic, symptomatic to advanced HIV /AIDS. These stages are defined by specific clinical conditions or symptoms too.

Category A (asymptomatic)

These are the asymptomatic, acute HIV with or without persistent generalized lymphadenopathy with CD4 count greater than 500 cells / µL.

Category B (symptomatic)

These are the individuals with CD4 count between 200 and 499 cells / µL. They met at least one of the following criteria: pelvic inflammatory disease (PID), bacillary angiomatosis,

oropharyngeal candidiasis, vulvovaginal candidiasis, cervical dysplasia, cervical carcinoma, oral hairy leukoplakia, Herpes zoster, idiopathic thrombopenic purpura, peripheral neuropathy, fever or diarrhea lasting for more than one month.

Category C (AIDS indicated conditions)

These are the individuals with CD4 count less than 200 cells / µL. They met many of the following criteria: recurrent puemonia, pelvic inflammatory disease (PID), and candida of the trachea, bronchi or lungs, cervical carcinoma, coccidioidomycosis, cryptococcosis, cryptosporidiosis, cytomegalovirus disease, encephalopathy, HIV related simplex with chronic ulcers for more than one month or bronchitis, pneumonitis or esophagitis, Histoplasmosis, isosporiasis with chronic intestinal disorder for more than one month, Kaposi sarcoma, lymphoma, mycobacterium avium complex (MAC) or mycobacterium kankasi, mycobacterium tuberculosis, Pneumocystis jiveci pneumonia (PCP), progressive multifocal leukoencephalopathy (PML), salmonella septicaemia, toxoplasmosis of brain, wasting syndrome for more than one month or chronic weakness and fever for more than one month.

2.1.7 Types, groups and subtypes of HIV

HIV is highly variable virus which mutates very readily. This means that the many different strains of HIV may be present within the body of a single infected person (Wainberg, 2004). HIV is classified, on the basis of its genetic differences and similarities into types, groups and subtypes. There are two types of HIV; HIV-1 and HIV-2. Both types are transmitted by sexual contract, from mother to child and through blood, and the AIDS produced by them is clinically indistinguishable. (Coovadia, 2004). However, it seems that HIV-2 is less easily transmitted and the period between initial infection and illness is longer in the case of HIV-2 (Talor *et al*, 2008). Worldwide, the predominant virus is HIV-1 while HIV-2 is concentrated only in West Africa (Stebbing *et al*, 2004).

The strains of HIV-1 can be classified into four groups, namely; the major group-HIV-M; the outlier group-HIV-O and the new groups-HIV-N and HIV-P. These four groups may

represent four separate introductions of simian immunodeficiency virus into humans (Plantier, 2009).

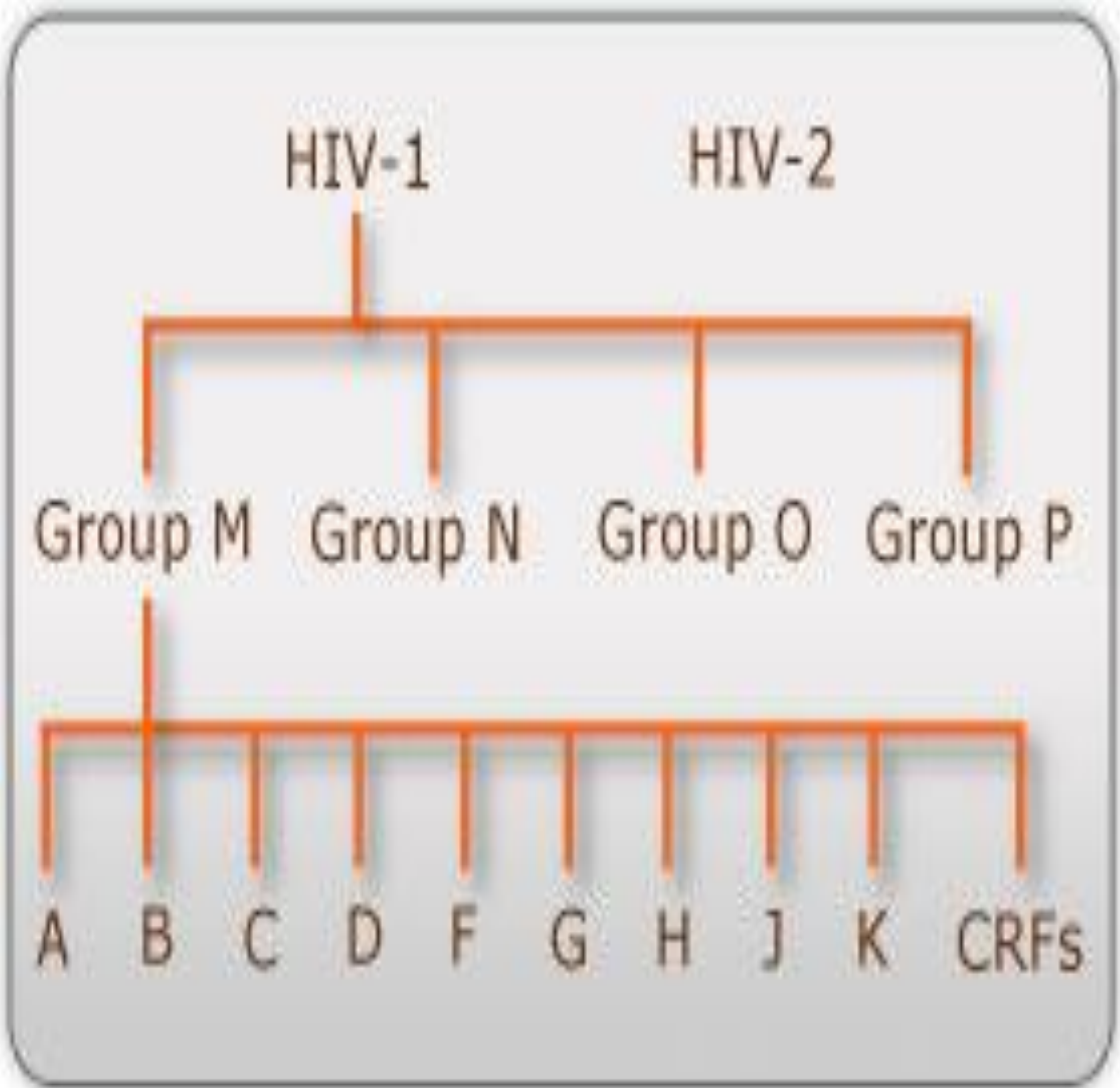


Figure 2.4: Diagram of HIV types, groups and subtypes

Ninety (90) % of HIV-1 infections belong to group M (Martin *et al,* 2000). Group 0 appears to be restricted to west-central Africa and group N discovered in 1998 in Cameroon is extremely rare (Talor *et al,* 2008).

Within the group M there are known to be at least nine genetically district subtypes of HIV-1. These subtypes include; A, B, C, D, F, G, H, J and K. Occasionally, two viruses of different subtypes can infect an individual, these often result in creation of new hybrid virus (viral sex) known as "circulating recombinant forms" or CRF**s**. For example, the CRF A/B is a mixture of subtypes A and B. (Burke, 1997; Talor *et al,* 2008). Also, both inter and intra-clad multiple infection have been reported in HIV individuals, and this kind of transmission has been associated with more disease progression (Weiss, 2008).

2.1.8 Transmission of HIV

HIV is transmitted by three main routes: sexual contact, exposure to body fluids or tissues and from mother to child during pregnancy, delivery or breastfeeding known as vertical transmission (William and Steven, 2007). There is no risk of acquiring HIV if exposed to faeces, nasal secretions, saliva, sputum, sweat, tears, urine or vomit unless these are contaminated with blood (kripke, 2007). It is possible to be co-infected by more than one strain of HIV-a condition known as HIV superinfection (Kuyi and Cornelissen, 2007).

Sexual contact: The majority of HIV infections are acquired through sexual relations. Sexual transmission can occur when infected sexual secretion of one partner come in contact with the genital oral or sexual mucous membranes of another. The risk of transmission of HIV can be increased when there is sexual assault; sexually transmitted infection. This is because it disrupts the normal epithelial barrier causing genital ulceration and/or micro-ulceration. In addition, accumulation of pools of HIV infected semen in the vagina sections of the female may also predispose to HIV infection (Hader *et al,* 2001; Hariri and McKenna, 2007). Women are more susceptible to HIV infection due to hormonal changes, vaginal microbial

ecology and physiology and a higher prevalence of sexually transmitted disease (Lamptey, 2002).

As regards unprotected heterosexual contacts, estimates of the risk of HIV transmission per sexual act appear to be four to ten times higher in low – income countries than in high-income countries (Boily *et al,* 2009). In low- income countries, the risk of female- to- male transmission is estimated as 0.38% per act and of male – to –female transmission as 0.30% per act; the equivalent estimates for high – income countries are 0.04% per act for female- to-male transmission and 0.08% per act for male- to- female transmission. The risk of transmission from anal intercourse is especially high, estimated as 1.4- 1.7% per act in both heterosexual and homosexual contacts (Beyrer *et al,* 2012). The risk from receiving oral sex has been described as 'nearly nil' (Sturchler, 2006), however, a few cases have been reported. The per-act risk is estimated at 0 – 0.04% for receptive oral intercourse (Dosekun and Fox, 2010). While the risk of transmission from oral sex is relatively low, it is still present (Yu and Vajdy, 2010).

The risk of transmission increases in the presence of many sexually transmitted infections and genital ulcers (Ng *et al,* 2011). Genital ulcers appear to increase the risk approximately fivefold. Other sexually transmitted infections such as gonorrhea, Chlamydia, trichomoniasis and bacterial vaginosis are associated with smaller increases in risk of transmission (Dosekun and Fox, 2010).

Rough sex can be a factor associated with an increased risk of transmission (Klimas *et al,* 2008). Sexual assault is also believed to carry an increases risk of HIV transmission as condoms are rarely worn, physical trauma to the vagina or rectum is likely and there may be a greater risk of concurrent sexually transmitted infections (Draughon and Sheridan, 2012).

Body fluids: The second most frequent mode of HIV transmission is through blood and blood products (Willian and Steven, 2007). Blood–borne transmission can be through needle-sharing during intravenous drug use, needle stick injury, transfusion of contaminated blood or blood product or medical injections with unsterilized equipment. The risk from sharing a

needle during drug injection is between 0.63 and 2.4% per act, with an average of of 0.8% (Baggaley *et al*, 2001; Lawn, 2004; Baggaley *et al*, 2006). The risk of acquiring HIV from a needle stick from an HIV infected person is estimated as o.3% (about 1 in 333) per act (Kripke, 2007).

People giving or receiving tattoos, piercings and scarification are theoretically at risk of infection but no confirmed cases have been documented. It is not possible for mosquitoes or other insects to transmit HIV (CDC, 2012).

Mother- to- child: This is the third most common way in which HIV is transmitted worldwide. HIV can be transmitted from mother to the child during pregnancy, during delivery or through breast milk (Coovadia, 2004). In the absence of treatment, the risk of transmission before or during birth is around 20% and in those who breastfeed is 35%. In 2008, mother- to- child transmission was about 90% of cases of HIV (Coutsoudis *et al*, 2010).

2.1.9 Prevention

Sexual contact: Consistent condom use reduces the risk of HIV transmission by approximately 80% over the long term (Crosby and Bounse, 2012). When condoms are used consistently by a couple in which one person is infected, the rate of HIV infection is less than 1% per year (WHO, 2003). There is some evidence to suggest that female condoms may provide an equivalent level of protection (Gallo *et al,* 2012). Application of a vaginal gel containing tenofovir (a reverse transcriptase inhibitor) immediately before sex seems to reduce infection rates by approximately 40% among African women (Celum and Baeten, 2012). Circumcision in sub-Sahran Africa reduces the acquisition of HIV by heterosexual men by 38% and 66% over 24 months (Siegfried *et al*, 2009). Based on these studies, the World Health Organization and UNAIDS both recommended male circumcision as a method of preventing female- to- male HIV transmission in 2007 (WHO 2007; UNAIDS 2007).

Pre-exposure: Treating people with HIV whose CD4 count ≥ 350 cells/µL with antiretroviral protects 96% of their partners from infection. This is about a 10 to 20 fold reduction in

transmission risk (Chou *et al,* 2012). Pre-exposure prophylaxis with a daily dose of the medications tenovir, with or without emtricitabine, is effective in a number of groups including men who have sex with men, couples where one is HIV positive and young heterosexuals in Africa (Celum and Baeten, 2012). Universal precautions within the health care environment are believed to be effective in decreasing the risk of HIV (Kurth et al, 2011).

Post-exposure: A course of antiretrovirals administered within 48 to 72 hours after exposure to HIV positive blood or genital secretions is referred to as post-exposure prophylaxis. The use of the single agent zidovudine reduces the risk of subsequent HIV infection fivefold following a needle stick injury. Treatment is recommended after assault is violated with a known HIV positive individual who refuses to delare his HIV status (Linden, 2011). Current treatment regimes typically use lopinavir/ ritonavir and lamivudine/ zidovudine or emtricitabine/ tenofovir and may decrease the risk further. The duration of treatment is usually four weeks (Young *et al*, 2007) and is frequently associated with adverse effects (with zidovudine in about 70% of cases, including nausea in 24%, fatique in 22%, emotional distress in 13% and headache in 9%) (Kripke, 2007).

Mother–to-child: Programs to prevent the vertical transmission of HIV can reduce rates of transmission by 92 – 99% (Kurth *et al*, 2011). This primarily involves the use of a combination of antiviral medications during pregnancy and after birth in the infant and potentially includes bottle feeding rather than breast feeding (Siegfried *et al*, 2011).

Vaccination: As of 2012, there was no effective vaccine for HIV or AIDS. A single trial of the vaccine RV 144 published in 2009 found a partial reduction in the risk of transmission of about 30%, stimulating some hope in the research community of developing a truly effective vaccine. Further trials of the RV 144 vaccine are on-going (Reynell and Trkola, 2012).

2.1.10 Diagnosis of HIV/AIDS

Many HIV- positive people are unaware that they are infected with the virus. HIV infection is diagnosed using clinical signs and symptoms as well as specific laboratory tests. The clinical

signs and symptoms are according to the WHO staging system for HIV infection and disease. Staging the severity of patient's disease allows health care professionals to determine the best time to initiate treatment with antiretroviral (ARV) therapy. HIV/AIDS is diagnosed in the laboratory by antibody tests, virology tests and stimmunology.

Antibody Tests: An antibody test detects the antibody produced during the immune response to HIV. This antibody test is cheap, easy to perform and accessible. These tests include HIV rapid tests, Enzyme Linked Immunosorbent Assay (ELISA) and Western Blot (Greenwald *et al*, 2006).

Virology Tests: Virology tests directly detect the presence of the virus in the blood sample. These tests detect the RNA, DNA or protein of the virus. They include:

HIV-RNA PCR: This is used to monitor the response to HIV treatment. It is a quantitative test and indicates the viral load in a patient's blood sample (Jones *et al*, 2005).

HIV-DNA PCR: This is used to detect HIV DNA material that is located inside the host cell. DNA PCR can be run using either serum or dried blood spots. It is a 'gold standard' of early infant diagnosis. The test is very sensitive and specific and can detect the virus in the first months of life (Scott, 2005).

Ultrasensitive P24 antigen assay: This is used to detect HIV in the blood. The antigen P24 is a major core protein on HIV that can be found either free in blood stream of HIV infected persons or bound to antigen P24 antibody (Patton *et al*, 2006).

Antibody tests in children younger than 18 months are typically inaccurate due to the continued presence of maternal antibodies (Kellerman and Essajee, 2010). Thus HIV infection can only be diagnosed by PCR testing for HIV RNA or DNA or via testing for the p24 antigen

Stimmunology: This is the test used to detect HIV within a week or during the window period. The test is also known as smartube. It involves the incubation of HIV primed lymphocytes which is transformed into plasma cell which secrete HIV specific antibodies (Tehuda-Cohen, 2011).

2.1.11 Biochemical changes in HIV infection

The progression and replication of HIV in cells and the use of Antiretroviral therapy have shown to result in the impairment of some tissues and organs which include the liver, gonads, kidney and the pancreas leading to some biochemical changes. Previous study has documented that the liver is an important site of HIV replication (Szczech *et al*, 2007) and that antiretroviral agents have some adverse effect on the liver resulting to elevated transaminases levels (Dieterich *et al*, 2004).

Raised serum Circulating immune complex (CIC) was found to be increased in asymptomatic HIV/AIDS individuals and this rise was observed to correlate with disease progression (Onyenekwe *et al*, 2007).

Also, low ascorbic acid levels were observed in asymptomatic HIV/AIDS individuals (Onyenekwe *et al*, 2007) hence it serves as a nutritional marker in treating these patients since as an antioxidant; it mops up free radicals in the system. It has been reported that the administration of vitamin C to HIV individuals can significantly reduce protease inhibitor agent (Slain *et al*, 2003). Serum anti- BCG and PCV have been mostly affected by HIV infection even in cases of malaria HIV co-morbidity in endemic areas (Onyenekwe *et al,* 2008).

Other metabolic complications in HIV, including dyslipidemia, insulin resistance, and altered fat distribution have been reported to be common in adults infected with HIV who are receiving ART. These complications have been reported to have increased in these patients' risk of cardiovascular disease (Fontas *et al*, 2004).

Men with HIV infection were reported to have hypocholesterolaemia with and without hypertriglyceridaemia (Anastos *et al*, 2007). The mechanism for hypocholesterolaemia in HIV is not known. A pattern of hyperlipidaemia i.e elevated levels of total cholesterol, LDL-C, triglycerides and a reduced level of HDL-C has been observed in patients treated with Protease inhibitors (Khiangte *et al,* 2007). Hypertriglyceridaemia was the first dyslipidaemia to be reported in HIV infected patient but other lipid abnormalities such as

hypocholesterolaemia or hypo HDL cholesterolaemia have also been reported (Khiangte *et al,* 2007).

Serum albumin has been shown to be useful in predicting mortality due to HIV infection in infected subjects. Some studies have it that albumin has a positive correlation with $CD4^+$ Tell count. Low serum levels of albumin have been observed in HIV infected individuals (Onyenekwe *et al*, 2007, Grahamet *et al*, 2007). The impact on the serum albumin was ascribed to be as a result of metabolic imbalance favouring catabolism as a result of inadequate protein synthesis due to chronic state of infection (Joseph *et al,* 2000). Serum albumin levels were observed to be a good predictor of the severity of HIV disease in individuals who are not taking antiretroviral therapy and can also indicate the extent of a patient's response to HIV treatment (Olawuni *et al*, 2006). Nevertheless, albumin levels are not a marker of HIV infection status, they have been found to be a strong predictor of mortality in HIV positive adults and children (Michael, 2005).

2.1.12 Treatment and management of HIV/AIDS

Antiretroviral drugs are medications for the treatment of infection by retroviruses, primarily HIV. Treatment also includes prevention and active treatment of opportunistic infections. There are three antiretroviral drugs used, each of which targets a different part of the virus multiplication cycle. These are the Nucleoside reverse transcriptase inhibitors (NRTIs), the Protease inhibitors (PIs), and the Non-nucleoside reverse transcriptase inhibitors (NNRTIs) (UNAIDS, 2003).

The nucleoside reverse transcriptase inhibitors inhibit the HIV reverse transcriptase enzyme competitively and terminate synthesis of DNA chains. They are Lamivudine (3TC or emtricitabine (FTC)), Zidovudine (AZT) (tenofovir or, Didanosine), Zalcibabine (ddC), abacavir and stavudine (d4T). Zidovudine was the first pharmacologic agent that was developed in the nineties that had significant effectiveness for treatment of HIV infection. Zidovudine has been documented to prolong the lives of treated patients by decreasing the incidence and severity of opportunistic infections, by partly suppressing HIV replication, and

also increasing CD4 lymphocyte counts. (Hirsch & D'Aquila, 1993). Common side effects of AZT include nausea, headache, changes in body fat, and discoloration of finger and toe nails. More severe side effects include anaemia and bone marrow suppression (Cohn, 1997). More so, NRTIs are effective in persons who cannot tolerate zidovudine as evidenced by decreased CD4 lymphocyte counts and increased in HIV plasma viremia (Deeks, 1998).

In addition to the NRTIs, non-nucleoside reverse transcriptase inhibitors (NNRTIs) have been developed for the treatment of HIV infection. These drugs act via non-competitive binding to a hydrophobic pocket close to the active site of the reverse transcriptase enzyme of HIV. These drugs include nevirapine, delavirdine, and efavirenz. They are most useful when either is used in combination with other antiretroviral agents. The major complication with use of these NNRTIs is skin rash (Cohn, 1997) and hepatic enzyme elevation (Dieterich & colleagues, 2004).

Protease inhibitors are synthetic analogues of the HIV protein that block the action of HIV-protease to interfere with viral replication. Protease inhibitors also function by decreasing CD4 lymphocyte apoptosis through decreased CD4 interleukin-1ß-converting enzyme (ICE, or caspase1) expression. They exhibit gastrointestinal symptoms like nausea, vomiting, and diarrhea (Yanovski *et al*, 1999).

In vitro studies have demonstrated that protease inhibitors can inhibit lipogenesis and adipocyte differentiation, (Dowell *et al,* 2000) stimulate lipolysis, (Lenhard *et al*, 2000) and impair sterol regulatory enhancer–binding protein 1 (SREBP1) nuclear localization (Caron *et al*, 2003). The nucleoside analogue linked most strongly to lipoatrophy is stavudine, particularly when used in combination with didanosine (Dube *et al*, 2002; Mallon *et al,* 2003).

Protease inhibitors, most notably ritonavir were seen to have increased hepatic triglyceride synthesis and plasma triglyceride levels (Lenhard *et al,* 2000). A newer protease inhibitor, atazanavir, does not appear to have this effect (Murphy *et al,* 2003). Protease inhibitors also tend to increase total cholesterol levels, but this effect also varies among the individual drugs

in this class (Periard, 1999). Alterations in apolipoprotein B occur in patients receiving combination therapy (with a nucleoside analogue and a protease inhibitor): notably, there is an increase in LDL 2 concentration; an increase in apolipoprotein B; and a shift toward triglyceride-rich very-low-density lipoprotein (Schmitz *et al*, 2001). Also, the levels of lipoprotein particles containing apolipoprotein C-III and apolipoprotein E have been reported to have increased in protease-inhibitor-treated patients (Bonnet, 2001).

Drug intolerance and drug toxicity are significant problems for all drugs used to treat HIV infection. These adverse effects are manifested by abdominal pain, nausea, or vomiting, lipodystrophy, dyslipidemia, diarhoea, insulin resistance and an increase in cardiovascular risks (Burgoyne & Tan, 2008; Barbaro and Barbarini, 2011). Other additional side effects are osteoporosis, neuropathy, cancers and nephropathy. These conditions result from the effect from the viral infection (Lewis, 2000), the metabolic side effects of antiretroviral therapy and in addition to genetic host factors (Aberg, 2009).These side effects if not treated have a mortality rate of about 50% (Squires, 2001). It is needful to carefully monitor Patients for signs and symptoms of these complications (Squires, 2001).

Current HIV treatment consists of the use of highly active antiretroviral therapy (HAART) which is a multiple drug combinations of antiretroviral agents is now used for HIV therapy. Typical regimens consist of two nucleoside analogue reverse transcriptase inhibitors (NARTIs or NRTIs) plus either a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor (WHO, 2003; 2013; 2015). The standard goals of HAART include improvement in the patient's quality of life, reduction in complications and reduction of HIV viraemia below the limit of detection, but it does not cure the patient of HIV nor does it prevent the return of HIV. Once treatment is stopped, HAART resistance set in (Dybul *et al,* 2002; WHO, 2003; 2013; 2015).

When to start antiretroviral therapy is subject to debate (Sax and Baden, 2009). The World Health Organization, European guildelines and the United States recommends antiretrovirals in all adolescents, adults and pregnant women with a CD4 count less than 350/μL or those

with symptoms regardless of CD4 count (Vogel *et al*, 2010). This is supported by the fact that beginning treatment at this level reduces the risk of death (Siegfrie *et al,* 2010). While another school of thought proffered that to be eligible for treatment, a person living with HIV/AIDS must have a $CD4^{+}$ T cell count of less than 200. Once treatment is commenced, there should not be a break in the supply of line of drugs in order not to encourage viral drug resistance (Cooney, 2002; Burgoyne & Tan, 2008; Vogel *et al*, 2010).

Treatment recommendations for children are slightly different from those for adults. In the developing world, as of 2010, 23% of children who were in need of treatment had access. Both the World Health Organization and the United States recommend treatment for all children less than twelve months of age. The United States recommends treatment in those between one year and five years of age and in those more than five years with CD4 counts less than 500/µL (WHO, 2010).

Benefits of treatment include a decreased risk of progression to AIDS and a decreased risk of death (Sterne *et al*, 2009). In the developing world, treatment also improves physical and mental health (Beard *et al*, 2009). With treatment, there is 70% reduced risk of acquiring tuberculosis. Additional benefits include a decreased risk of transmission of the disease to sexual partners and a decrease in mother- to- child transmission. The effectiveness of treatment depends to a large part on compliance. Reasons for non-adherence include poor access to medical care, inadequate social supports, mental illness and drug abuse (Malta *et al*, 2008). The complexity of treatment regimens (due to pill numbers and dosing frequency) and adverse effects may reduce adherence (Nachega *et al,* 2011) even though cost is an important issue with some medications (Orsi and d'almeida, 2010).

Measures to prevent opportunistic infections are effective in many people with HIV/AIDS. Treatment with antiretroviral therapy reduces the risk of developing additional opportunistic infections (Montessori *et al*, 2004). Vaccination against hepatitis A and B is advised for all people at risk of HIV before they become infected; however, it may also be given after infection (Laurence, 2006).

2.1.13 Prognosis

HIV/AIDS has become a chronic rather than an acutely fatal disease in many areas of the world (Knoll *et al*, 2007). Prognosis varies between people and both the CD4 count and viral load are useful for predicting outcomes. Without treatment, average survival time after infection with HIV is estimated to be 9 to 11 years, depending on the HIV subtype. After the diagnosis of AIDS, if treatment is not available, survival ranges between 6 and 19 months (Morgan *et al*, 2002). HAART and appropriate prevention of opportunistic infections reduces the death rate by 80% and raises the life expectancy for a newly diagnosed young adult to 20 -50 years (Schackman *et al*, 2006). This is between two thirds and nearly that of the general population (Sighem *et al*, 2010). If treatment is started late in the infection, prognosis is not as good: for example, if treatment is begun following the diagnosis of AIDS, life expectancy is 10- 40 years. Half of infants born with HIV die before two years of age without treatment.

The primary causes of death from HIV/AIDS are opportunistic infections and cancer, both of which are frequently the result of the progressive failure of the immune system (Cheung et al, 2005). Risk of cancer appears to increase once the CD4 count is below 500/ML. The rate of clinical disease progression varies widely between individuals and has been shown to be affected by a number of factors such as a person's susceptibility and immune function, their access to health care, the presence of co- infection and the particular strain (s) of the virus involved (Lawn, 2004).

Tuberculosis co- infection is one of the leading causes of sickness and death in those with HIV/AIDS being present in a third of all HIV infected people and causing 25% of HIV related deaths. Hepatitis C is another very common co- infection where each disease increases the progression of the other. The two most common cancers associated with HIV/AIDS are Kaposi sarcoma and AIDS-related non-hodgkin lymphoma (Cheung *et al,* 2005).

Even with anti- retroviral treatment, over the long term HIV-infected people may experience neurocognitive disorders, osteoporosis, neuropathy, cancers, nephropathy and cardiovascular

disease. It is not clear whether these conditions result from the HIV infection itself or are adverse effects of treatment.

2.2 Review of malaria

2.2.1 Causes and transmission of malaria

Malaria is a debilitating and life threatening protozoan disease in Human commonly caused by any of the four species of the genus Plasmodium namely *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale and Plasmodium malariae.*- a mosquito-borne infectious disease. It is naturally transmitted by the bite of a female anopheles mosquito (Singh *et al,* 2004).

The signs and symptoms of malaria begin about 7 – 25 days after infection (Fairhurst *et al,* 2010). The classical symptoms of malaria is febrile paroxysm which is a cyclical occurrence of sudden coldness followed by rigor, then fever and sweating which last for six hours, occurring every two days (tertian fever) for *P. vivax* and *P ovale* infection and every three days (quartan fever) in *P. malarae.* Severe malaria is mainly caused by *P. falciparum* infection and it usually arises between 6-14 days after infection (Baton *et al*, 2005; Olupot-Olupot and Maitland, 2013). *P. falciparum* infection can cause recurrent fever every 36- 48 hours or a less pronounced and almost continuous fever (Bartoloni and Zammarchi, 2012).

These parasites spent about two weeks and several months in the liver and multiply within red blood cells causing symptoms like fever and headache. In severe cases, the disease worsens leading to hallucinations, coma and even death (Snow *et al*, 2005). Other signs and symptoms of malaria include shivering, arthralgia, vomiting, anaemia, jaundice, anaemia, vomiting, shilvering, joint pain, headache, pyrexia, haemoglobinaemia, renal damage and convulsion (Beare *et al,* 2006; Bartoloni and Zammarchi, 2012; Nadjm and Behrens 2012). Consequences of severe malaria include coma and death but if not treated especially in young children splenomegaly, severe headache, cerebral ischemia, haemogloblinuria, hepatomegaly and renal failure may occur (Trampus *et al*, 2003).

Heavy parasitisation by *P. falciparum* may result in pernicious malaria which is a compex of life-threatening complication that may supervene in acute malaria. Pernicious malaria is of three types: septicaemic malaria, algid malaria and cerebral malaria (Arora and Arora, 2006). Septicaemic malaria is characterized by a high degree of prostration; there is high continous fever with the involvement of various organs. Algid malaria resembles surgical shock with cold clammy skin, peripheral circulatory failure and profound shock. Patients may also develop vomiting and diarhoea or dysentery. In cerebral malaria, the capillaries of the brain are plugged with parasitized red blood cells, each cell containing malaria pigment. In holoendemic ares of malaria, cerebral malaria is characterized by neurological symptoms such as abdominal posturing, conjugate gaze palsy, opisthotonus, seizures or coma (Bartoloni and Zammarchi, 2012).

Cerebral malaria is associated with retinal whitening which is a useful clinical sign in distinguishing malaria from other causes of fever (Maude *et al,* 2009).

2.2.2 Pathogenesis of malaria

Malaria in human is developed in two phases-exoerythrocytic and erythrocytic phase. The exoerythrocytic phase involves the infection of the liver, whereas erythrocytic phase involves infection of the erythrocyte. The plasmodium parasite enters the host skin through the bite of mosquito. The sporozoites in the mosquito's saliva enter the bloodstream and migrate to the liver. Within 30 minutes, the sporozoites infect hepatocytes, multiply asexually and symptomatically for a period of 6-15 days. They differentiate into thousands of merozoites, rupture from their host cells, they escape into the blood and infect red cells, thus marking the genesis of the erythrocytic stage of the life cycle (Bledsoe, 2005).

During blood meal of an infected host, a mosquito picks up gamatocytes which are developed from merozoites. Fertilization and sexual recombination of the parasite occurs in the mosquito's gut, to produce sporozoites that travel to the salivary glands of the mosquito for infection into an individual. So mosquito is the definitive host of the infection.

The malaria parasite evade host immune response using mechanisms such as antigenic variation, splenic- dependent regulation of parasite genes encoding structural proteins and adhesive molecules on the erythrocyte surface that are involved in adherence to the endothelium as well as low immunogenicity of conserved parasite peptides that are targets of antibodies able to interfere with parasite survival (Benson *et al*, 2001; Riley *et al*, 2013).

During the erythrocytic cycle, soluble products of *Plasmodium spp*. Known as malaria toxins direct the systemic release of pro-inflammatory cytokines such as Tumor Necrosis Factor alpha (TNF-α) which act on many other cellular components such as endothelium. Parasite antigens also stimulate T cells to directly secrete or induce production of cytokines from other cells. Evidence has shown that incubation of blood mononuclear cells with parasitized erythrocytes can drive proliferation of these T cells even when the parasitemia is as low as one parasite per microlitre of blood (Miller *et al,* 1994). Because many of these T cells secrete interferon γ (IFN-γ) and other cytokines and can facilitate the production of IFN-α by monocytes, they are involved in disease pathogenesis. The parasite- dependent activation of T cells and mononuclear phagocytes leads to the manifestation of the disease (Miller *et al,* 1994).

2.2.3 Epidemiology of malaria

Malaria is widespread in tropical and subtropical region, including parts of the Americans (22 countries), Asia and Africa. Each year, about 350 – 500 million cases of malaria are recorded with about one and three million people died of the infection, the majority of the death are children in sub-Sahara Africa (Krogstad, 2007; CDC report, 2008).

It is estimated that 3.3 billion people (half the world's population) live in area at risk of malaria transmission in 109 countries and territories; 35 countries (30 countries in sub-Sahara Africa and 5 in Asia) account for 98% of global malaria deaths. WHO estimates that in 2008 malaria caused 190 – 311 million clinical episodes of malaria and one million deaths world-wide. Approximately 90% of the malaria deaths world-wide occur in Africa. Malaria is the fifth cause of death from infectious diseases world-wide and the second cause of death from

infectious diseases in Africa after HIV/AIDS (CDC report, 2009; WHO 2013). In 2012, Malaria caused an estimated 627,000 deaths with an uncertainty range of 473,000 to 789,000 (WHO, 3013).

Malaria is the most common cause of out-patient visits in all age categories in Nigeria and it is the major cause of morbidity and mortality in all parts of Nigeria (FMH, 2005). It is the second leading cause of death after HIV/AIDS (WHO, 2013). Malaria is a major public health burden in Nigeria; it is responsible for more hospital cases and deaths than any other country in the world (WHO, 2013). It is the largest killer of children under the age of five years and has been estimated to kill 3000 children daily (WHO, 2012).

Nigeria is a country with a population of about 149 million of which about 97% are exposed to steady malaria transmission. There are five ecological strata from South to North that define vector specie dominance, seasonality and intensity of malaria transmission namely mangrove, swamps, rain forest, guinea-Sudan and sahel-Savana. Accordingly, the duration of the transmission reduces from south to north. The prevalence specie of malaria parasite is Plasmodium falciparum. 300 malaria related deaths are reported annually in children less than 5 years in Nigeria (National Malaria Control Programme, Nigeria Strategic Plan, 2009-2013).

2.2.4 Diagnosis of malaria

The World Health Organization recommends that before giving treatment, clinical malaria should be confirmed by parasite- based diagnosis and that presumptive diagnosis and treatment should only be considered when parasitological diagnosis is not possible (Orogade, 2012).

The diagnosis of malaria requires careful clinical examination and laboratory investigation (Orogade, 2012).The mainstay of malaria diagnosis has been the microscopic examination of blood. The thin and thick films are reliable in diagnosing the different types of *Plasmodium spp*. for more than 100 years (Moody, 2002).Thin films allow for species identification between the parasites appearance and are the best preserved in the preparation. The thick

films allow the microscopist to screen for a larger volume of blood and are about eleven times more sensitive than the thin film but both films are used to make a definitive diagnosis (Warhurst *et al*, 1996).

The limitation of microscopy prompted research and development of reliable, easy to perform tests such as the Rapid Diagnostic Test (RDTs) to detect the presence of malaria parasites at levels of accuracy compared to skilled microscopists (Kim *et al,* 2013). The test is called immunochromatographic test/malaria rapid diagnostic test/antigen-captive assay or dipsticks (McCutchan *et al,* 2008). The RDTS are antigen detecting diagnostic tests which detect the histidine rich protein 2 (HRP2) Plasmodium Lactate dehydrogenase (pLDH) which are usually produced during the erythrocytic cycle (Orogado, 2012). At present, different RDTs are available for malaria diagnosis. The performance varies with the environment it is employed in, the geographic location, the disease prevalence and the prevalent parasite species. The sensivity of antigen dectecting diagnostic tests, decline with low parasite densities (<300 -500µL) (Kim *et al,* 2008). The successful implementation of RDT has been bedeviled by poor product performance, inadequate methods to determine the quality of products and a lack of emphasis and capacity to deal with these (Bell *et al*, 2006).

Molecular tools are highly sensitive in detecting low levels of infections and accurately detecting species of malaria parasites (Okell *et al*, 2009). Modern methods such as Polymerase Chain Reactions or monoclonal antibody panels may be use to distinguish between two species of malaria (McCutchan *et al,* 2008). Many of these molecular tools for malaria diagnosis range from conventional Polymerase Chain Reactions (PCR)- based assays, real- time PCR assay and isothermal assays (Lucchi *et al*, 2013).

Real- time PCR assays depend on the use of fluorophores or DNA intercalating fluorescent dyes. Sequence- specific oligonucleotide probes are dual labeled with a fluorescent dye and quencher. A less complicated real-time PCR technique uses non-specific DNA intercalating dyes such as SYBR Green, DYTO-9 and Calcein which emit fluorescence signals when bound to double stranded DNA but they do not allow for multiplexing. Other alternatives for

the detection of real- time PGR assays uses the direct labeling of one of the primers with a single fluorophore in a manner that facilitates self quenching without the need of a quencher. These self-qunching primers facilitate the use of real-time PCR without the need for internal dual- labeled sequence specific probes (Nazarenko 2006; Lucchi *et al*, 2013).

Nested PGR is the most widely used reference standard in malaria diagnostic research. It is useful tool for epidemiological studies (WHO, 2013). Nested PGR reduces non-specific binding in products due to the amplification of unexpected primer binding sites. It involves the use of two set of primers used in two successive runs of polymerase chain reaction. The second set is used to amplify a secondary target within the first run product (Fuehrer *et al*, 2011).

Current research is ongoing to seek for information for the WHO to review its current recommendation of exclusive use of microscopy for the confirmation of malaria species and detection of gametocytes (WHO, 2013).

2.2.5 Biochemical changes in malaria

Reports have it that in clinical case of malaria, anaemia has been shown to be a prominent factor that results from the destruction of infected red cells by plasmodium at the reticuloendothelial system and haemolysis of infected red cells. Also, a negative association between malaria parasite density and packed cell volume has been observed which suggests the possibility that anaemia may occur with further degree of parasiteamia (Onyenekwe *et al*, 2005).

A high incidence rate of asymptomatic malaria parasiteamia has been observed amongst pregnant women in Nigeria (Onyenekwe *et al,* 2002). Hence, this high incidence rate of malaria parasitaemia in pregnant women can lead to anaemia, increased mortality and low birth weight.

Leukocytosis, leucopenia, elevated fibrinogen degradation, elevated erythrocytes sedimentation rate and thrombocytopenia are examples of haematological changes that have been reported in malaria infection (Miller *et al,* 1994).

Complicated malaria especially in children can lead to liver and kidney dysfunctions with severe jaundice and increased liver enzymes. In acute attack especially in children, spleen and liver enlargement may be observed (Schofield and Gran, 2005).

2.2.6 Treatment and management of malaria

Antimalaria therapies drugs designed to prevent or cure malaria. Antimalarial drugs previously used as monotherapy were Quinine, Chloroqiune, Amodiaquine, Pyrimethamine, Proguanil, Sulfonamide, Mefliquine, Atovaquone, Primaquine, Artemisinin, Halofantrine, Doxycycline, Clindamycin etc.

Quinine is an alkaloid that acts as a blood schizonticidal and weak gametocide against *Plasmodium vivax* and *Plasmodium malariae*. It acts by inhibiting the hemozoin biocrystallization, thus facilitating an aggregation of cytotoxic heme. It is effective and widely used in the treatment of acute cases of severe *P. falciparum* (WHO, 2010).

Chloroqiune is a 4-aminoquinolone compound with a complicated and still unclear mechanism of action. It is believed to reach high concentrations in the vacuoles of the parasite, which, due to its alkaline nature, raises the internal pH. It controls the conversion of toxic heme to hemozoin by inhibiting the biocrystallization of hemozoin, thus poisoning the parasite through excess levels of toxicity. The most significant level of activity found is against all forms of the schizonts and the gametocytes of *P. vivax, P. malariae, P. ovale* as well as the immature gametocytes of *P. falciparum*. It is also has anti-pyretic and anti-inflammatory effect when used to treat *P. vivax* infections, and still remain useful when resistance is more widespread (WHO, 2010).

Amodiaquine is a 4-aminoquinolone anti-malarial drug similar in structure and mechanism of action to chloroquine. Amodiaquine has tended to be administered in areas of chloroquine resistance while some patients prefer its tendency to cause less itching than chloroquine. Amodiaquine is now available in a combined formulation with artesunate (ASAQ) and is among the artemisinin-combination therapies recommended by the World Health

Organisation. Combination with sulfadoxine=pyrimethamine is no longer recommended (WHO, 2010).

Pyrimethamine is used in the treatment of uncomplicated malaria. It is particularly useful in cases of chloroquine-resistant *P. falciparum* strains when combined with sulfadoxine. It acts by inhibiting dihydrofolate reductase in the parasite thus preventing the biosynthesis of purines and pyrimidines, thereby halting the processes of DNA replication, cell division and reproduction. It acts primarily on the schizonts during the erythrocytic phase, and nowadays is only used in concert with a sulfonamide (WHO, 2010; WHO, 2013).

Proguanil (chloroguanide) is a biguanide; a synthetic derivative of pyrimidine. It has many mechanisms of action but primarily is mediated through conversion to the active metabolite cycloguanil. This inhibits the malarial dihydrofolate reductase enzyme. Its most prominent effect is on the primary tissue stages of *P. falciparum, P. vivax* and *P. ovale*. It has a weak blood schizonticidal activity and is not recommended for therapy of acute infection WHO, 2013). Sulfadoxine and sulfamethoxypyridazine are specific inhibitors of the enzyme dihydropteroate synthetase in the tetrahydrofolate synthesis pathway of malaria parasites. They are structural analogs of *p*-aminobenzoic acid (PABA) and compete with PABA to block its conversion to dihydrofolic acid. Sulfonamides act on the schizont stages of the erythrocytic (asexual) cycle. When administered alone sulfonamides are not efficacious in treating malaria but co-administration with the antifolate pyrimethamine, most commonly as fixed-dose sulfadoxine-pyrimethamine (Fansidar), produces synergistic effects sufficient to cure sensitive strains of malaria(WHO, 2010; WHO, 2013).

Mefloquine was developed during the Vietnam War and is chemically related to quinine. It was developed to protect American troops against multi-drug resistant *P. falciparum*. It is a very potent blood schizonticide with a long half-life. It is thought to act by forming toxic heme complexes that damage parasitic food vacuoles. It is now used solely for the prevention of resistant strains of *P. falciparum* despite being effective against *P. vivax*, *P. ovale* and *P.*

marlariae. Mefloquine is effective in prophylaxis and for acute therapy (WHO, 2010; WHO, 2013).

Atovaquone is available in combination with proguanil under the name Malarone. It is commonly used in prophylaxis by travellers and used to treat falciparum malaria in developed countries. A liquid oral suspension of Atovaquone is available under the name Mepron (WHO, 2010; WHO, 2013).

Primaquine is a highly active 8-aminoquinolone that is used in treating all types of malaria infection. It is most effective against gametocytes but also acts on hypnozoites, blood schizonticytes and the dormant plasmodia in *P. vivax* and *P. ovale*. It is the only known drug to cure both relapsing malaria infections and acute cases. The mechanism of action is not fully understood but it is thought to block oxidative metabolism in Plasmodia (WHO, 2010; Baird *et al*, 2012; Eziefula *et al*, 2014).

Artemisinin is a Chinese herb that has been used in the treatment of fevers. The active compound is named artemsinin which has proven to be effective against all forms of multi-drug resistant *P. falciparum*, thus every care is taken to ensure compliance and adherence together with other behaviors associated with the development of resistance. It is also only given in combination with other anti-malarials. It has a very rapid action and the vast majority of acute patients treated show significant improvement within 1–3 days of receiving treatment (WHO, 2006; WHO, 2010; Douglas *et al*, 2010).

It has demonstrated the fastest clearance of all anti-malarials currently used and acts primarily on the trophozite phase, thus preventing progression of the disease. Semi-synthetic artemisinin derivatives (e.g. artesunate, artemether) are easier to use than the parent compound and are converted rapidly once in the body to the active compound dihydroartemesinin (WHO, 2010).

Halofantrine is a phenanthrene methanol, chemically related to Quinine and acts acting as a blood schizonticide effective against all plasmodium parasites. Its mechanism of action is similar to other anti-malarials. Cytotoxic complexes are formed with ferritoporphyrin XI that

cause plasmodial membrane damage. Despite being effective against drug resistant parasites, halofantrine is not commonly used in the treatment of malaria due to its high cost. Lumefantrine is a relative of halofantrine that is used in some combination antimalarial regimens (de Villers *et al*, 2008; WHO, 2010).

Doxycycline is a tetracycline compound derived from oxytetracycline. It is a bacteriostatic agent that acts to inhibit the process of protein synthesis. Doxycycline is used primarily for chemoprophylaxis in areas where chloroquine resistance exists. It can also be used in combination with quinine to treat resistant cases of *P. falciparum* but has a very slow action in acute malaria, and should not be used as monotherapy (WHO, 2010; WHO, 2013).

Clindamycin is a derivative of lincomycin, with a slow action against blood schizonticides. It is only used in combination with quinine in the treatment of acute cases of resistant *P. falciparum* infections and not as a prophylactic. Being more expensive and toxic than the other antibiotic alternatives, it is used only in cases where the Tetracyclines are contraindicated especially in children (WHO, 2010).

Current practice in treating cases of malaria is based on the concept of combination therapy, since this offers several advantages, including reduced risk of treatment failure, reduced risk of developing resistance, enhanced convenience, and reduced side-effects. Prompt parasitological confirmation by microscopy, or alternatively by rapid diagnostic tests, is recommended in all patients suspected of malaria before treatment is started (WHO, 2010; WHO, 2013).

WHO recommends Artemisinin-based combination therapies (ACTs) for the treatment of ubcomplicated malaria caused by the *Plasmodium falciparum* parasite. By combining two active ingredients with different mechanisms of action, ACTs are the most effective antimalarial medicines available today.Artemisinin and its deravatives must not be used as oral monotherapy, as this promotes the development of artemisinin resistance (WHO, 2010; WHO, 2013).

WHO currently recommended five ACTs for use against *Plasmodium falciparum* malaria. Fat acting artemisinin-based compounds are combined with a drug from a different class which includes lumefantrine, mefloquine, amodiaquine, sulfadoxine, pyrimethamine, piperaquine, chlorproguanil/ dapsone. Artemether/lumefantrine was the first fixed dose artemisinin-based combination therapy recommended by WHO for the treatment of uncomplicated malaria caused by *Plasmodium falciparum* (WHO, 2010; WHO, 2013).

Artemether is a methyl ether derivative of dihydroartemesinin. It is similar to artemesinin in mode of action but demonstrates a reduced ability as a hypnozoiticidal compound, instead acting more significantly to decrease gametocyte carriage. Similar restrictions are in place, as with artemesinin, to prevent the development of resistance, therefore it is only used in combination therapy for severe acute cases of drug-resistant *P. falciparum* (WHO, 2010; WHO, 2013).

Artesunate is a hemisuccinate derivative of the active metabolite dihydroartemisin. Currently it is the most frequently used of all the artemesinin-type drugs. Its only effect is mediated through a reduction in the gametocyte transmission. It is used in combination therapy and is effective in cases of uncomplicated *P. falciparum* (WHO, 2010; WHO, 2013).

Dihydroartemisinin is the active metabolite to which artemesinin is reduced. It is the most effective artemesinin compound and the least stable. It has a strong blood schizonticidal action and reduces gametocyte transmission. It is used for therapeutic treatment of cases of resistant and uncomplicated *P. falciparum*. Arteether is an ethyl ether derivative of dihydroartemisinin. It is used in combination therapy for cases of uncomplicated resistant P. falciparum (WHO, 2010; WHO, 2013).

2.2.7 HIV and malaria co-infection

Malaria is a life threatening disease that cause major health problem in the tropics and subtropics. Out of the four forms of malaria parasites in human, *P. falciparum* is the most prevalent in sub- Sahara Africa (Krogstad, 2007).

Malaria and HIV-1 and 2 are the most common infections in sub-Saharan Africa and it is estimated that 38 million Africans are infected with HIV-1 (UNAIDS, 2004), whereas 300 million to 500 million suffer from malaria each year (WHO, 2009). Researchers have observed co-mobidity in both pathogens in Africa (Whitworth, 2000; Laufer *et al,* 2006). Abu-Raddad *et al,* (2006) observed that the dual infection of HIV and malaria have both fuels the spread of both diseases in sub-Sahara Africa.

P. falciparum has been shown to stimulate HIV-1 replication through the production of cytokines by activated lymphocytes (Xiao & Co, 1998; Froebel *et al*, 2004). *P. falciparum* also has been found to increase the potential reservoir for HIV in the placenta by increasing the number of chemokine receptor5 macrophages (Tkachuk *et al*, 2001). Onyenekwe *et al,* (2007) in their work observed almost a triple- fold prevalence of *P. falciparum* in symptomatic HIV subjects that were infected with the malaria disease.

An important study from Malawi showed that HIV-1 plasma viral loads were significantly higher in patients with malaria infection than in those without, and these levels remained higher for up to 10 weeks after treatment (Kublin *et al*, 2005). This increase in viral load of such patients may be due to the association of HIV and malaria. Since infection with HIV causes progressive cellular immunosuppression, and any resulting impairment in the immune response to malaria might also be associated with failure to prevent infection or to suppress parasitemia. This study suggests that malaria may speed the progression of HIV disease.

This study was supported by a study from Uganda ((Mermin *et al,* 2006; Gasasira *et al*, 2006: Kamya *et al*, 2006) and Nigeria (Onyenekwe *et al*, 2008) that showed an increase in CD4 cell decline in symptomatic HIV subjects with malaria infectivity. Onyenekwe *et al*, (2008) have shown a significant biochemical changes in serum iron and albumin levels in symptomatic HIV subjects with malaria co- infection. Both parameters were found to be low in the study. Onifade *et al* (2007) observed among children infected with HIV a high infectivity of malaria more than those infected in adults in Ondo, Nigeria.

HIV-related immunosuppression has been suggested to increase the rates of malaria infection and clinical malaria disease, but does not increase the rates of complicated malaria (Patnaik *et al*, 2005; Laufer *et al*, 2006). It has been reported that the odds of parasitemia and risk of malarial fever increase with decreasing CD4 count and increasing viral load. These findings suggest that HIV infection may interfere with parasite control and more importantly, may cause the loss of antitoxic immunity, which protects persons with parasitemia from clinical disease. The disease burden has been found to be similar in all age groups because preexisting antimalarial immunity is limited. As a result, malarial fever rates are a direct function of parasite transmission rates. Thus, HIV co-infection has its impact on disease presentation, with an increased risk of complication, severe malaria and death (Grimwede *et al*, 2004; Cohen *et al*, 2005.

A study was carried out in malaria infected children and adults. The researcher observed severe malaria in the children which was reported to have caused multi-organ dysfunction and was the predominant reason for mortality in the children of study. They also observed elevated cardiac enzymes and cardiac involvement in some of these children studied and isolated reports of myocardial dysfunction in adults with malaria alone during the course of study (Poddutoor *et al,* 2010). Ehrhardt *et al (*2004; 2005) have shown that cardiac enzymes are elevated in complicated malaria.

2.2.8 Cardiovascular disease in HIV on ART

In a study, myocardial infarction, hyperinsulinemia, hyperglycemia, insulin resistance, lactic acidosis, osteonecrosis and osteoporosis were observed in HIV subjects on antiretroviral therapy (Carr, 2000; Behrens and Schmidt 2005). In an earlier study, Friis *et al* (2003; DAD report, 2004) showed that old age, current or former smoking, previous cardiovascular disease, male sex, hypercholesterolemia, hypertriglyceridemia and diabetes mellitus were associated with an increased risk of myocardial infarction. DAD report, (2004) also found

that the relative risk of cardiovascular disease increased as the duration of antiretroviral therapy increased.

The increased risk of developing heart disease in HIV individuals may be due to the direct effects of the human virus on the heart (Malnick and Goland, 1998), the chronic inflammatory effect of the virus itself on the myocardium (Lewis, 2000, Aberg, 2009) and the presence of autoantibodies (Malnick and Goland, 1998).

In antiretroviral therapy, replacement of a protease inhibitor with nevirapine, efavirenz, or abacavir has been reported to have effectively reduce total cholesterol (Martinez *et al,* 2003; Moyle *et al*, 2003), LDL cholesterol (Moyle *et al,* 2003) and triglyceride level (Moyle *et al*, 2003) and increase HDL cholesterol levels (Franssen *et al*, 2009). Also, body-fat changes tended to improve six months after a switch from a protease inhibitor to nevirapine (Barriero *et al*, 2000).

2. 2.9 Effect of HIV on lipid metabolism

Normally fat tissue in the body removes lipids from the blood, stores and releases them when they are needed for energy use. Rasheed *et al* (2008) presented the first direct evidence that HIV replication alone in human T- cells with or without any influence of antiviral drugs or other factors, can stimulate the production of cellular enzymes and proteins that enhance fatty acids synthesis, increase the quantity of LDL, secrete TGs, alter the lipid transport, metabolism and oxidize lipids. One of the most essential biological processes involved in dyslipidemia and lipodystrophy syndrome is the accumulation of lipids and disproportionate distribution of tissue-associated fats due to the enhanced fatty acid synthesis. HIV infection has been found to have profound impact on the adipocytes thereby disabling it from storing most lipids mainly triglycerides (Broxmeyer, 2004).

Also, the fat tissue in HIV positive individuals is less sensitive to insulin (insulin resistance) which maintains the body’s fat stores; as a result, their bodies break down fat for energy

instead of sugars, which causes increased levels of fat in the blood circulation as well as hyperinsulinemia (Carr *et al,* 2000). Other metabolic complications in HIV infection include lactic acidosis, osteoporosis and osteonecrosis (Carr *et al*, 2000).

2.3 CD4 count in HIV infection and management

There are three major types of lymphocyte namely T cells, B cells and natural killer (NK) cells. NK cells are a part of innate immune system that play a major role in defending the host from both tumors and virally infected cells. Lymphocytes are distinguished from other leukocytes by having a deeply staining nucleus which may be eccentric in location, and a relatively small amount of cytoplasm (Charles *et al*, 2001). It is impossible to distinguish between T cells and B cells in a peripheral blood smear, normally, flow cytometry testing is used for specific lymphocyte population counts (Abbas and Lichtman, 2006; Berrington *et al,* 2005).

The lymphocytes are involved in adaptive immunity and they differentiate further after exposure to an antigen to effector and memory lymphocytes. Effector lymphocytes function to eliminate the antigen, either by releasing antibodies (in the case of B cells), cytotoxic granules (cytotoxic T cells) or by signaling to other cells of the immune system (helper T cells/ $CD4^{+}$counts). The memory cells remain in the peripheral tissues and circulation for an extended time ready to respond to the same antigen upon future exposure (Abbas and Lichtman, 2006).

CD4 (cluster of differentiation 4) is a glycoprotein found on the surface of immune cells such as T helper cells, monocytes, macrophages and dendritic cells. It was discovered in the late 1970s and was originally known as leu-3 and T4 (after the OKT4 monoclonal antibody that reacted with it) before being named CD4 in 1984 (Alain, 1984). In humans, the CD4 protein is encoded by the CD4 gene (Ansari-Lari *et al*, 1996).

$CD4^{+}$ T cells are white blood cells that are an essential part of the human immune system. They are often referred to as CD4 cells, T helper cells or T4 cells. They are called helper cells

because one of their main roles is to send signals to other types of immune cells. CD4 cells send the signal and CD8 cells destroy and kill the infection/ virus (Brady *et al*, 1993).

CD4 is an aco-receptor that assists the T cell receptor (TCR) in communicating with an antigen- presenting cell. Using its intracellular domain, CD4 amplifies the signal generated by the TCR by recruiting an enzyme, the tyrosine kinase, which is essential for activating many molecular components of the signaling cascade of an activated T cell. CD4 also interacts directly with MHC class 11 molecules on the surface of the antigen- presenting cell using its extracellular domain (Ryu *et al*, 1994).

The acquired immunodeficiency syndrome (AIDS), is a fatal illness caused by HIV that breaks down the body's immune system, infects CD4 lymphocytes, deplete them and progressively lead to AIDS (Rasool *et al*, 2008). HIV infects $CD4^+$ T cell counts which may be by direct cytotoxicity, thereby, limiting the regeneration of T-cell in the thymus (Smith *et al*, 2000).

The $CD4^+$ T cell counts are the primary target of HIV infection because of the affinity of the virus to the CD4 surface marker. Infection with HIV leads to a progressive impairment of cellular functions, which is characterized by a gradual decline in peripheral blood $CD4^+$ T cell counts levels and a wide variety of opportunistic, viral, bacterial, protozoal and fungal infections and to certain malignancies also (Khiangte *et al*, 2007).

A decrease in the total CD4 lymphocyte count below 500/µL may lead to the development of clinical AIDS, and a drop below 200/µL not only defines AIDS, but also indicates a high probability for the development of AIDS-related opportunistic infections and/or neoplasms. The risk for death from HIV infection above the 200/µL CD4 level is low (Bozzette *et al*, 1995).

Several different CD4 tests are used along with viral load test to evaluate HIV/AIDS patients' medical conditions and monitor their responses to treatment. CD4 blood tests measure the amount of CD4 $^+$T-cells that are circulating in the blood while viral load tests determine how many viral particles are present in the blood. HIV patients who are otherwise healthy and

symptom-free should have their CD4 cell count and viral load tested about two to four times a year. But, symptomatic patients should be tested more frequently to evaluate both the risk of opportunistic infections and the response to HIV drug treatments,

An earlier study reported that a decrease of $CD4^+$ T counts due to HIV disease progression was accompanied by a decrease in total cholesterol, HDL, LDL and significant increases in both triglyceride, and VLDL levels (Palanisamy *et al,* 2008).

Antiretroviral therapy is a drug that acts on the patient's CD4 cell count by suppressing HIV. The drugs interfere with HIV's ability to replicate and infect new cells, which slows down the progression of the disease. CD4 cell count tests can indicate whether or not the treatment is working. In response to successful antiretroviral therapy, the CD4 count typically increases by more than 50 cells per microliter of blood within a few weeks and then increases by 50 – 100 cells per year thereafter until a threshold is reached. Thresholds vary among patients. Antiretroviral therapy is taken indefinitely to control the virus.

CD4 test help healthcare providers determine whether the treatment needs to be changed. The test also indicates when preventive treatment against opportunistic infections may be necessary. The CD4 count also guides decision making in determining when to stop prophylaxis against opportunistic infections with patients whose CD4 counts rise in response. Many factors can influence the CD4 results, these include; stress levels, the time of the day, other infections and nutritional status.

2.4 The Heart

In Human, the heart is a muscular organ, about the size of a fist. It pumps blood throughout the blood vessels to various parts of the body by repeated, rhythmic contractions. It is composed of cardiac muscle and connective tissue. The average human heart, beating at 72 beats per minute and pumps approximately 4.7-5.7 litres of blood per minute. It weighs approximately 250 to 300 grams in females and 300 to 350 grams in males (Fausto, 2005).

The heart is usually situated in the thoracic cavity, surrounded by the lungs and underneath the sternum. The heart is fed by the coronary circulation and its wall is made up of three layers-the pericardium an outermost layer; which contains serous fluid that reduces friction during heart contractions. The myocardium is the muscular middle layer and the endocardium which lines inside the heart (Maton *et al*, 1993).

2.4.1 The physiology of the Heart

The physiology of the heart involves what happens between systole and diastole. In systole, cardiac tissue is contracting to push blood out of the chamber and in diastole; cardiac tissue is relaxing to the chamber to fill with blood. Deoxygenated blood returning from the body enters the heart from superior and inferior vana cava. The blood enters the right atrium, pump through triscuspid valve into right ventricle, the blood passes through pulmonary semilunar valve into the pulmonary trunk, which carries blood to lungs, releases carbon dioxide and absorbs oxygen, which returns from lungs to the heart through pulmonary veins, from the pulmonary veins, blood enters the heart again in the left atrium, it contracts through bicuspid (mitral) valve into left ventricular aortic semilunar valve into the aorta and finally emptied into the systemic circulation and flows throughout the body, until the blood returns to the heart via vena cava and the cycle repeats (Jani and Rajkumar, 2006).

Some cardiac cells are self-excitable, contracting without any signal from the nervous system. Each of the cells has their own intrinsic contraction rhythm. A region of the human heart called the sinoatrial (SA) node, or pacemaker, sets the rate and timing at which all cardiac muscle cells contract. The SA node generates electrical impulses, which spread rapidly through the walls of the artria, causing both artria to contract in unison (Jani and Rajkumar, 2006).

The impulses also pass to the wall between the right atrium and the right ventricle, a relay point called the atrioventricular node, located in specialized muscle fibers called Purkinje fibers. Here, the impulses are delayed for about 0.1s before spreading to the walls of the ventricle. The delay ensures that the artria empty completely before the ventricles contract.

The Purkinje fibers, then conduct the signals to the apex of the heart along and throughout the ventricular walls (Jani and Rajkumar, 2006).

This entire cycle, a single heart beat, lasts about 0.8 seconds. The impulses generated during the heart cycle produce electrical currents, which are conducted through body fluids to the skin, where they can be detected by electrodes and recorded as an electrocardiogram (ECG) (Jani and Rajkumar, 2006). The events related to the flow or blood pressure that occurs from the beginning of one heartbeat to the beginning of the next is called a cardiac cycle (Fausto, 2005).

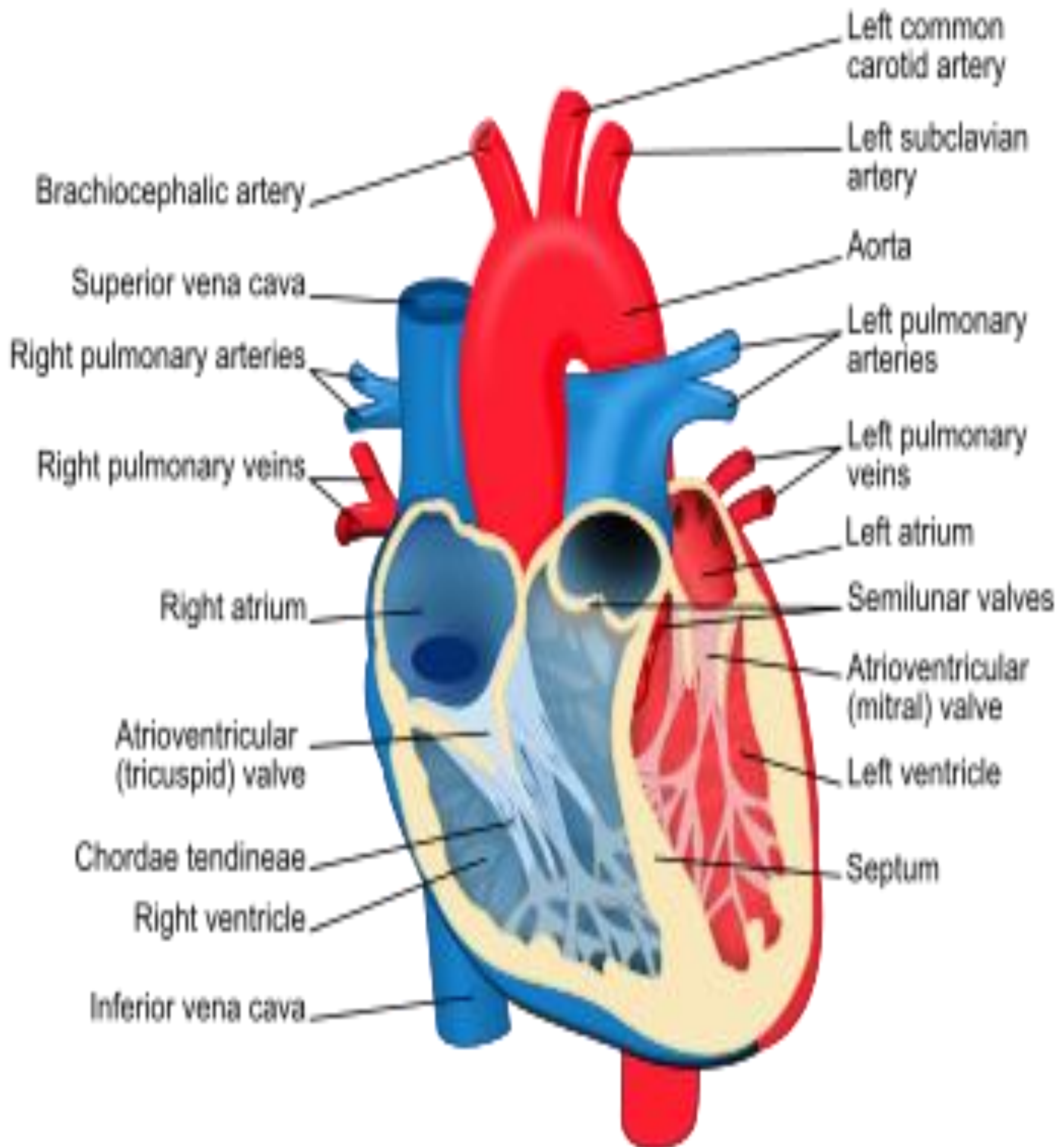


Figure 2.5: Diagram of the human heart from an anterior view. Blue components indicate deoxygenated blood pathways and red components indicate oxygenated pathways. (Adapted from Fausto, 2005).

2.4.2 Cardiovascular diseases

This is a class of diseases that involve the heart, the blood vessels or both (Maron *et al*, 1993).Cardiovascular disease refers to any disease that affects the cardiovascular system, mainly the cardiac disease, vascular diseases of the brain and kidney, and peripheral arterial disease (Kelly, 2010). The causes of cardiovascular disease are diverse but atherosclerosis

and hypertension are the most common (Dantas *et al,* 2012). Cardiovascular diseases are the leading cause of deaths worldwide (Mendis *et al*, 2011).

There are several risk factors that cause heart diseases: age, gender, tobacco use, physical inactivity, excessive alcohol consumption, unhealthy diet, obesity, family history of cardiovascular disease, hypertension, diabetes mellitus, hyperlipidemia, poverty and low educational status, and air pollution. Some of these risk factors, such as age, gender or family history, are non- modifiable; but, other cardiovascular risk factors such as lifestyle change, social change, drug treatment and prevention of hypertension, hyperlipidemia, and diabetes are modifiable (Shanthi *et al*, 2011; Micha *et al*, 2012; Finks *et al,* 2012).

Types of cardiovascular diseases include coronary artery disease also known as ischemic heart disease (Faxon, 2004; Bhatia and Sujata, 2010), cardiomyopathy (Richardson *et al,* 1996), hypertensive heart disease (Alegria-Ezquerra *et al*, 1996), heart failure (chronic heart failure) (McDonagh, 2011), pulmonary heart disease – a failure at the right side of the heart with respiratory system involvement (American Medical Network, 2010), cardiac dysrhythmias – abnormalities of heart rhythm (Mandel, 1995), Inflammatory heart disease- endocarditis- inflammatory cardiomegaly,-inflammatory cardiomyopathy (Kasper *et al*, 2005), valvular heart disease (Bono *et al*, 2006), cerebrovascular disease (WHO, 2009), peripheral arterial disease – disease of blood vessels that supply blood to the arms and legs (Joosten *et al*, 2012) etc.

2.4.3 Pathophysiology of cardiovascular diseases

The main cause of most cardiovascular diseases is atherosclerosis (Dantas *et al*, 2012), which is an abnormal built up of fat and other substances which form plague inside the arteries. In chronic hyperlipidaemia, atherosclerosis develops when LDL molecules become oxidized by free radicals from macrophages or enthothelial cells into oxidized LDL, which accumulates in the macrophages and other phagocytes, forming foam cells. The foam cells also known as “fatty streaks" clogged the artery, reducing the elasticity of the artery walls. A series of

reactions occur to repair the oxidized LDL. These white blood cells are not able to process the oxidized LDL and it ultimately grows, then rupture, depositing a greater amount of oxidized cholesterol into the artery wall. This triggers more white blood cells thereby continuing the cycle (Nicholas *et al,* 2006; Kumar and Fausto, 2010; Dantas *et al*, 2012). Eventually, the artery becomes inflamed. The cholesterol plaque causes the cover to enlarge and form a hard cover over the affected area. This hard cover causes narrowing of the artery which reduces the blood flow, thus leading to ischaemia. Atherosclerosis is most serious when it leads to reduced or blocked blood supply to the heart (causing angina or heart attack) or to the brain causing stroke (Nicholas *et al,* 2006; Kumar and Fausto, 2010; Dantas *et al*, 2012).

Most commonly the soft plaque suddenly ruptures causing the formation of a thrombus that will rapidly slow or stop blood flow, leading to death of the tissue fed by the artery in about 5 minutes. This catastrophic event is called an infarction. One of the most common recognized scenarios is called coronary thrombosis of a coronary artery, causing myocardial infarction (heart attack). The same process in an artery to the brain is commonly called stroke (Nicholas *et al,* 2006).

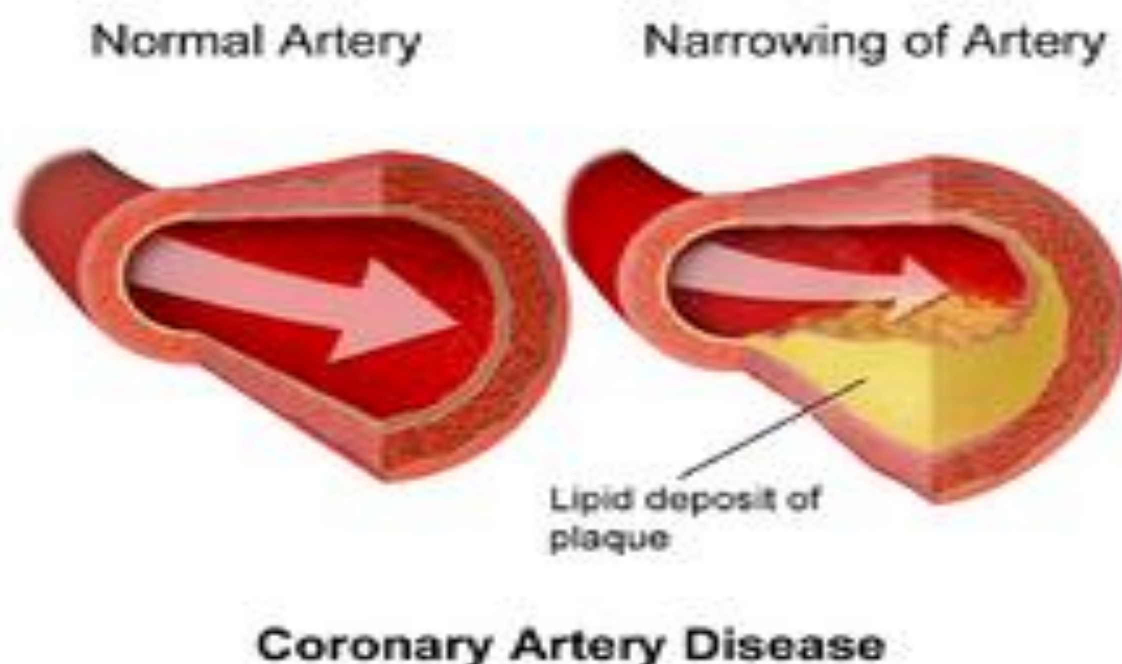
People with elevated level of fibrinogen are more likely to die twice to heart attack or stroke (Packard *et al*, 2000). This is because during injury, fibrinogen is converted to fibrin, which forms the structural matrix of a blood clot. So, increased level of fibrinogen has been reported to have increased blood clot formation, which often lead to thrombosis (Koenig, 1999).

Again, low level of vitamin K predisposes ageing in human to vascular calcification, causing atherosclerosis. Vitamin K regulates osteocalcin that directs calcium to the bones and keep it out of the arterial wall for possible calcification (Schurgers, 2007; Beulens, 2009).

Cardiovascular artery disease, in 2010 was the leading cause of death globally (Lozano *et al,* 2012). It is common in older ages, with male affected more often than females (Finegold *et al*, 2012). However, drugs such as statins have been reported to reduce the serum level of cholesterol as well as reduce the risk of coronary disease (Gutierret *et al,* 2012). Also,

antioxidants such as vitamin E, C, D and Niacin have been found to improve endothelial dysfunction and nitric oxide synthase activity (Carlson, 2005) which is required for healthy cardiovascular function (Nitenberg, 2006).

The diagnosis of heart disease is either by the use of blood test and non-blood test method such as electrography that measures the electrical activity of the heart through the skin; and others such as ultradound, echocardiography, radioisotope test, coronary angiography. The blood test is used to identify cardiac markers that are released in blood during heart muscle damage (Rao *et al,* 1999; Wang *et al,* 2006; Spence, 2006; Moyer, 2012; Maron *et al,* 2014).



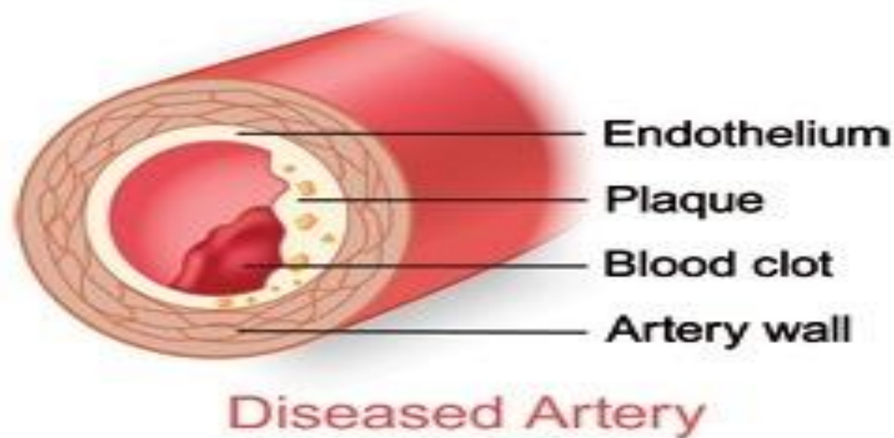


Figure 2.6: Diagram of coronary heart disease. (Adapted from Nicholas *et al,* 2006)

2.5 Cardiac biomarkers

There have been reports that individuals infected with HIV have increased risk of developing cardiopathy (Sudano *et al*, 2006). Cardiac biomarkers are biomarkers measured to evaluate heart function especially in cardiac diseases. They are released in blood during heart damage (Rao *et al*, 1999; Vasadevan *et al*, 2011).

2.5.1 Cardiac enzyme markers

An enzyme is a protein that catalyses one or more specific biochemical reactions. Generally, enzymes are present in cells at much higher concentrations than plasma (Crook, 2006)

2.5.1.1 Creatine kinase (CK)

Creatine-kinase is an enzyme that catalyses the conversion of creatine to creatine phosphate. Creatine consists of two protein subunits, M and B, which combine to form three isonzymes, namely: CK-BB (CK-1), CK-MB (CK-2), CK-MM (CK-3) (Vasudevan *et al*, 2011). CK-MM is an isoenzyme found in the skeletal and cardiac muscle and is detectable in the plasma of normal subjects. Most of the CK released after a myocardial infarction is the MM isoform which is found both in skeletal and myocardial muscle and has a longer half-life than the MB fraction (Isah, 2007; Vasudevan *et al*, 2011).

CK-MB accounts for about 35 per cent of the total CK activity in cardiac muscle and less than 5 per cent in skeletal muscle; its plasma activity is always high after myocardial infarction. There are two isoforms of MB, namely CK-MB1 and CK-MB2. The later is predominantly released from the myocardium. Creatine kinase –MB that is routinely assayed reflects the sum of the two isoforms. Normally, CK-MB predominates in plasma but after an acute myocardial infarction this is reversed (Isah, 2007; Vasudevan *et al*, 2011).

CK-BB is present in high concentrations in the brain and in the smooth muscle of the gastrointestinal and genital tracts (Crook, 2006). CK value starts to rise within 3-6 hours of infarction. Hence, CK estimation is very useful to detect early cases of MI. CK level is not increased in haemolysis or in congestive cardiac failure and therefore, CK have an advantage over LDH (Crook, 2006). Estimation of total CK is employed in muscular dystrophies and MB isoenzyme is estimated in myocardial infarction (Moss, 2001; Isah, 2007).

2.5.1.2 Aspartate transferase (AST)

Aspartate transferase was formally called serum glutamate oxaloacetate transaminase (SGOT). AST needs pyridoxal phosphate (vitamin B6) as co-enzyme. AST is present in higher concentrations in cells of cardiac and skeletal muscle, liver, kidney and erythrocytes. Damage to any of these tissues may increase plasma AST levels. AST is a marker of liver injury and shows moderate to drastic increase in parenchymal liver diseases like hepatitis and malignancies of live; measurement of plasma AST level is therefore not a specific marker for cardiac function (Vasudevan *et al*, 2011).

The sequence of changes in plasma AST levels after myocardial infarction have been reported to be similar to those of CK, both have between 24 – 48 hours peak rise after MI. It has been observed that a small MI does cause some hepatic congestion due to right-sided heart dysfunction and this may contribute to the rise of plasma AST activity. This is rarely a diagnostic problem because the increase in plasma AST activity following an infarction is usually much greater (Crook, 2006; Vasudevan *et al*, 2011). AST was used as a marker of

myocardial ischemia in olden days but troponins have replaced it as a diagnostic marker in schemic heart disease (Vasudevan *et al*, 2011)

2.5.1.3 Lactate dehydrogenase (LDH)

Lactate dehydrogenase catalyses the reversible interconversion of lactate to pyruvate. The enzyme is widely distributed in the body, with high concentrations in cells of cardiac and skeletal muscles, liver, kidney, brain and erythrocytes; measurement of plasma total LDH activity is therefore a non-specific marker of cell damage. So, damage to any of these tissues may increase plasma LDH levels (Crook, 2006; Isah, 2007).

Five main isoenzymes are detected by electrophoresis and are referred to as LDH1 to LDH5. LDH1, the fraction that migrates fastest towards the anode, predominates in cells of cardiac muscle, erythrocytes and kidney. The slowest moving isoenzyme, LDH5, is the most abundant form in the liver and in skeletal muscle (Moss, 2001; Crook 2006; Isah, 2007).

It is rarely necessary to quantitate LDH isoenzyme activity. A rise in LDH1 is most significant in the diagnosis of myocardial infarction. Total LDH is sometimes used in the diagnosis of a myocardial infarction, nervertheless; troponin has largely taken over this role from LDH. LDH is also a marker for certain tumours, for example, lymphomas and to help determine haemolysis (Moss, 2001; Crook, 2006).

In summary, CK, AST and LDH have been of established diagnostic criteria for the confirmation of suspected acute myocardial infarction (AMI). However, none of this enzyme is specific for the heart tissue. The advantage of CK over AST and LDH is that its distribution in the heart is confined to the myocardial tissue rather than to the connective tissue and blood elements of the heart. The specificity of CK has been enhanced by the measurement of the isoenzyme, CK-MB in blood (Moss, 2001; Crook, 2006).

2.5.2 Cardiac protein markers

2.5.2.1 Myoglobin

Myoglobin is a low molecular weight haem-containing protein that is found in both skeletal and cardiac muscle. Myoglobin is responsible for the oxygen deposition in muscle tissues.

Myoglobin is known as a marker of myocardial damage and it has been used for more than three decades (Kagen *et al*, 1997). Due to its low molecular weight, it is rapidly released from the myocardium upon damage and a typical rise occurs within 2 – 4 hours after the early diagnosis of acute myocardial infarction. This is useful for the early diagnosis of acute myocardial infarction, as this rise is generally earlier than that of the other presently used cardiac markers (Penttila *et al*, 2002). It appears in patients' blood 1 – 3 hours after onset of the symptoms, reaching peak level within 8 – 12 hours and return to normal at about 20 to 36 hours (Srinivas *et al,* 2001). Myoglobin has been found not to be so cardiac specific as troponin (Van Nieuwenhoven *et al,* 1995). A persistently normal myoglobin level may rule out heart muscle damage. But once elevated, an elevated troponin level is required to make a definitive diagnosis. Thus myoglobin is used together with other cardiac markers in clinical practice for better specificity in AMI diagnosis (Antman, 2007).

2.5.2.2 Troponin complex

Troponin complex is a protein playing an important role in the regulation of skeletal and cardiac muscle contraction. Troponin complex consists of three different subunits – troponin T (TnT), troponin I (TnI) and troponin C (TnC). TnT is a tropomyosin-binding subunit which regulates the interaction of troponin complex with thin filaments in the heart; TnI inhibits ATP-ase activity of acto-myosin; TnC is a Ca2+ - binding subunit, playing the main role in Ca2+ dependent regulation of muscle contraction (Sheehan and Vasikaran, 2001; Gomes *et al,* 2002;Vasudevan *et al,* 2011).

TnT and TnI in cardiac muscle are presented by forms different from those in skeletal muscles. Two isoforms of TnI and two isoforms of TnT are expressed in human skeletal muscle tissue (skTnI and skTnT). Only one tissue-specific isoform of TnI is described for cardiac muscle tissue (cTnI). No cardiac specific isoform is known for human TnC. TnC in human cardiac muscle tissue is presented by an isoform typical for slow skeletal muscle (Marston and Redwood, 2003).

cTnI is expressed only in myocardium. cTnT is probably less cardiac specific because it may be elevated in patients with chronic renal failure (Sheehan and Vasikaran, 2001; Sarko and Pollack, 2002). cTnI (Cummius *et al*, 1987) and later cTnT (Katus *et al*, 1989) were used as markers of cardiac cell death. Now both proteins are widely used for the diagnosis of acute myocardial infarction (AMI) and other heart diseases. Both markers can be detected in patient's blood 3-6hours after onset of the chest pain, reaching peak level within 16–30 hours. Elevated concentration of cTnI and cTnT in blood samples can be detected even 5–8 days after onset of the symptoms, making both proteins useful also for the late diagnosis of AMI (Hamm, 2001).

2.6 Basic physiology of lipid metabolism

2.6.1 Plasma lipoproteins: structure, nomenclature and occurrence

Plasma lipoproteins are made up of complexes of lipids and proteins which are called apolipoproteins. The lipoprotein lipids are of different lipid types that include cholesterylesters (CE), triglycerides (TG) and phospholipids with the exception of free cholesterol, (Ginsberg, 1998). The lipoproteins are made up of particles with differences in density, volume and in lipid and protein content. Various methods are available that can isolate different subclasses and are classified according to their densities and electrophoretic motilities. In order of decreasing densities, they are high density lipoprotein (HDL), low density lipoprotein (LDL), intermediate density lipoprotein (IDL), very low density lipoprotein (VLDL), and chylomicron. The plasma lipoproteins can be altered in structure and composition by various types of hyperlipidaemia depending upon the content of triglyceride or cholesterol (Fielding and Frayn, 2003).

2.6.2 Apolipoproteins

Apolipoproteins (apoproteins) are proteins that bind to lipids to form lipoproteins, whose main function is to transport lipids and are mediated with several biochemical steps associated with plasma lipid metabolism. The apolipoproteins are designated by Roman letters and numerals (as example apo A_1 and apo C_2) (Ginsberg, 1998). Apolipoproteins act

as the vehicles for solubilisation and transport of lipids in the plasma compartment. Many of the apolipoproteins contain determinants that regulate several activities that are essential to normal lipid metabolism. Apolipoproteins are important in maintaining the structural integrity and solubility of lipoproteins. Some apolipoproteins stimulate enzymes that degrade plasma lipids. Others contain the ligands that mediate the binding of lipoproteins to cell surface receptors; the binding is succeeded by the internalisation of all or part of a lipoprotein and the regulation of intracellular lipid synthesis (Mahley *et al*, 1984).

Lipoproteins are classified into five main groups based on their buoyant density, which inversely reflects their size. The greater the lipid: protein ratio, the larger their size and the lower the density. The lipoproteins are chylomicroms, very low density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) (Crook, 2006).

The chylomicrons are the largest and least dense lipoproteins and they transport exogenous lipid from the intestine to all cells. Very low density lipoproteins (VLDLs) are lipoproteins that transport endogenous lipid from liver to cells. Intermediate-density lipoproteins (IDLs) are transient particles that are formed during the conversion of VLDL to LDL, normally; they are not present in the plasma. Chylomicrons, VLDLs and IDLs are triglyceride rich and may show turbid appearance (lipaemic) if present in high concentrations (Crook, 2006).

Low density lipoproteins are formed from VLDLs and they carry cholesterol to cells of the body. LDL contains only apo B. It represents about 70 per cent of the total cholesterol concentration. LDL particles, by virtue of their small size, can infiltrate tissues by passive diffusion and may cause damage, as in atheroma formation within arterial walls. In other word, an elevated level of LDL may cause atherosclerosis (Crook, 2006).

High density lipoproteins are synthesized in both hepatic and intestinal cells as small nascent HDL particles that are rich in free cholesterol, phospholipids, apo A, predominantly apo A_2 and apo E. If the plasma concentration of VLDL or chylomicron is low, apo C is also carried in HDL but as the plasma concentrations of these lipoproteins rise, they take back apo C from

HDL. lecithin cholesterol acyl transferase (LCAT) (Philips *et al*, 1998), which is important in removing excess cholesterol from tissues is present on HDL and catalysis the esterification of free cholesterol which, most of this esterified cholesterol is transferred to LDL, VLDL and some may be stored within the core of the HDL particle and taken directly to the liver (Crook, 2006).

2.6.2.1 Apolipoprotein A

Apolipoprotein A has two major forms, apo A_I and apo $A_{2,}$. Apo A_I is the major apolipoprotein associated with HDL-C and constitutes about 70% of the total protein (Srivastava and Srivastava, 2000; Sakurabayeshi *et al*, 2001). Apo A_I also acts as a cofactor for lecithin cholesterol acyl transferase (LCAT), an enzyme responsible for forming cholesterol esters in plasma and plays an important role in the transportation of excess cholesterol from peripheral tissues and incorporating it into HDL for reverse transport to the liver, to be finally excreted (Philips *et al*, 1998; Betteridge and Morrel, 1999).

Deficiency of apo A_1 is associated with HDL deficiencies, including systemic non-neuropathic amyloidosis and Tangier disease- which is a consequence of an Apo A_1 catabolism defect, characterized by severe reduced plasma HDL-C concentration, abnormal HDL composition and accumulation of cholesterol esters in many body tissues. Patients with deficiency of apo A_1 have increase Coronary heart disease (Srivastava and Srivastava, 2000; Sakurabayeshi *et al*, 2001).

Apo A_1 may have a role in protection against Alzheimer's disease. Apo A_1 and apo E have been found to interact to modify triglyceride levels in coronary heart disease patients. Apo A_5 is a probable biochemical and genetic marker of increased triglyceride concentrations and also a risk factor of coronary disease in some populations (Oliveira *et al*, 2008).

Apolipoprotein A_2 is part of structural protein of HDL (Sakurabayeshi *et al*, 2001). Reports have it that Apo A_2 inhibits hepatic and lipoprotein lipase (LL) activities. This effect tends to increase plasma TG and reduce plasma HDL (Kalopissis and Chambaz, 2000).

2.6.2.2 Apolipoprotein B

Apolipoprotein B is the major structural apolipoprotein in VLDL, LDL and chylomicrons. The most important function is the transport of rich triglycerides from liver and intestine to other tissues. Apo B exists in two forms, apo B_{48} and apo B_{100}. Apo B_{48} is the most component of chylomicrons, is synthesized in the intestine, where it is complexes with dietary TG and free cholesterol absorbed from the gut lumen to form chylomicron particles. Apo B_{100} is the most important component of LDL, is synthesized in the liver and moved into the plasma as part of IDL and VLDL particles. Apo B is essential for the binding of LDL particles to the LDL receptor and thus LDL absorbs cholesterol. An excess of apo B-containing particles is a main trigger in the atherogenic process (Talmud *et al,* 2002).

The concentration of plasma apo B particles is highly correlated with the level of non-HDL cholesterol, which is defined as total cholesterol (TC) minus HDL-C (Ballantyne *et al,* 2002). As HDL is known to be protective against cardiovascular risk, non-HDL-C reflects the fraction of blood cholesterol that is not contained in atheroprotective lipoproteins (EPDT report, 2002). Non-HDL-C has been found to predict nonfatal myocardial infarction (MI) and angina pectoris (Bittner *et al*, 2002). However, apo B has been found to be a better predictor of risk than non-HDL-C (Walldius *et al*, 2001).

Apo B /apo A1 ratios are thought to be a better marker of risk of vascular disease and a better guide to the adequacy of statin treatment than any cholesterol index (Walldius *et al*, 2001). Some reports have suggested that non-fasting plasma apo A_1: apo B ratio may be a useful measure of cardiovascular risk and was superior to any of the cholesterol ratios for estimation of the risk of acute myocardial infarction in all ethnic groups, in both sexes, and at all ages (McQueen *et al*, 2008).

Hyperbetalipoproteinemia is characterized by increased LDL Apo B_{100} concentrations with normal or moderately increased concentrations of LDL-C. The ratio of LDL to Apo B_{100} is therefore reduced in these patients. Defects in the Apo B structure or lipoproteins containing Apo B prevent the secretion of triglycerides rich intestinal and hepatic lipoproteins; this

disorder occurs in abetalipoproteinemia or homozygous hypobetalipoproteinemia (Sakurabayeshi *et al*, 2001; Schonfeld *et al*, 2005).

Abetalipoproteinaemia is a rare autosomal recessive disorder that interferes with the normal absorption of fat and fat-soluble vitamins. It is caused by a deficiency of apo B48 and apo B100. It is also associated with absent of LDL and VLDL. Clinical features include fat malabsorption, progressive ataxia, acanthocytic red blood cells and retinitis pigmentosa. Death usually occurs before the age of 30 years while hypobetalipoproteinaemia is a genetic disorder that can be caused by a mutation in the apo B gene. Hypobetalipoproteinaemia is associated with low levels of plasma cholesterol and LDL. (Schonfeld *et al*, 2005).

2.6.2.3 Apolipoprotein C

The C apolipoproteins (C_1, C_2 and C_3) are surface components of chylomicrons, VLDL and HDL (Betteridge and Morrel, 1999; Sakurabayeshi *et al*, 2001). It appears that the liver is the major site of synthesis of the Apo C proteins, with the intestine contributing a minor portion.There are major subtypes of apo C that alter the metabolism of TG-rich lipoproteins, principally IDL and VLDL, in a variety of complex ways (Chong and Bachenheimer, 2000; Jong and Havekes, 2000). Apolipoprotein C_I has an inhibitory action on the uptake of VLDL via hepatic receptors (Jong and Havekes, 2000). It is the major plasma inhibitor of cholesteryl ester transfer protein (CETP) and it interferes directly with fatty acid uptake (Shachter, 2001). Apo C_2 is present in plasma at a concentration of nearly 0.04%. Its main function appear to be associated with its ability to act as a cofactor in activating lipoprotein lipase (LL), an enzyme that hydrolysis the TGs in the lipoprotein, a lipolytic process that liberates fatty acids from the lipoprotein particle and makes them available for uptake in the periphery, mainly by adipose tissue or muscle (Brewer, 1999).

Deficiency or defective Apo C_2, reduces the activity of the LPL enzyme, impairs chylomicrons catabolism and increases plasma TGs. Those affected by this disorder have less than 10% of normal concentration of Apo C_2, the minimum amount needed for normal LPL

activity (Sakurabayeshi *et al*, 2001). Homozygous hereditary deficiency of Apo C_2 leads to a hyperchylomicronemic syndrome which is very similar to hereditary LPL deficiency. The heterozygous state for one mutant Apo C_2 alleles, when associated with Apo E4 also is associated with hyperglyceridemia (Sakurabayeshi *et al*, 2001).

Apo C_3 is the most abundant Apo C in plasma approximately 0.04g/l. Its plays an important role in controlling plasma TG metabolism and in determining the plasma concentration of potentially atherogenic triglycerides – rich lipoprotein (TRL). Apo C_3 inhibits the lipolysis of TRL and interferes with TRL clearance from the circulation (Jong and Havekes, 2000; Shachter, 2001). It inhibits the hepatic uptake of TRL and their remnants and also inhibits the activity of Lipoprotein lipase and hepatic lipase. Plasma concentration of Apo C_3 is positively correlated with the level of plasma TGs and Apo C_3 production is increased in patients with hypertriglyceridemia (Sakurabayeshi *et al*, 2001). High levels of apo C_3 have been associated with increased risk of atherogenesis and cardiovascular events (Sacks *et al,* 2000).

2.6.2.4. Apolipoprotein D

Apolipoprotein D is primarily associated with HDL, but its role in lipid metabolism has yet to be defined (Rassart *et al*, 2000).

2.6.2.5. Apolipoprotein E

Apo E is an important component of plasma lipoproteins and influences lipoprotein metabolism through its action as a receptor ligand by binding to a specific receptor on liver cells and peripheral cells. It is mainly produced by the liver and secreted as a glycoprotein. In humans, Apo E presents a genetic polymorphism; it exists as 3 major isoforms- Apo E2, E3 and E4 (Sakurabayeshi *et al*, 2001; Verghese *et al*, 2011).

Apo E is found primarily in VLDL, IDL and chylomicrons (Betteridge and Morrel, 1999). It is essential for the normal catabolism of triglyceride-rich lipoprotein constituents (Eichner *et al*, 2002; Singh *et al*, 2002). It plays a central role in the metabolism of chylomicrons and

VLDL remnants; regulates and facilitates lipid protein uptake in the liver through interaction of chylomicron remnants and binding of VLDL remnants to the LDL receptor. It also plays a role in the activation of several lipolytic enzymes- hepatic lipase and lipoprotein lipase and LCAT (Sakurabayeshi *et al*, 2001).

There is increasing evidence that it protects against atherogenesis through a variety of mechanisms (Davignon *et al,* 1999; Larkin *et al,* 2000). There have been reports on the association between apo E and neurodegenerative conditions such as multiple sclerosis and Alzheimer's disease (Fazekes *et al,* 2011). There are also convincing evidence linking the apo E genotype to risk of cerebral amyloid angiopathy (Verghese *et al*, 2011).

So, Lipids parameters such as elevated levels of IDL, VLDL and TG are associated with increased cardiovascular risk, as well as low levels of HDL (Talmud *et al,* 2002). Also, the ratio of total cholesterol and HDL is one of the best lipoprotein indices in predicting cardiovascular risk in the general population (Lewington *et al*, 2007). It has been reported that in some patients with cardiovascular disorder, normal LDL-cholesterol and total cholesterol levels have been observed. It has been suggested that apo B and apo A1 or its ratio be measured so to significantly improve the assessment of cardiovascular risk (Talmud *et al,* 2002). There is evidence that the measurement of various forms of apolipoproteins may improve the prediction of the risk of cardiovascular disease (Talmud *et al,* 2002). (Sakurabayeshi *et al*, 2001).

2.6.3 Intracellular lipid synthesis and lipoprotein assembly

The major secreted lipoproteins are VLDL which is assembled in the liver, and the chylomicron which is derived from the intestine. Both lipoproteins undergo remodelling in the plasma compartment, thus remnants or mature forms or circulating particles are found under fasting conditions (Seed *et al*, 1994). The assembly of lipoproteins containing apo-B is a complex process that occurs in the lumen of the endoplasmic reticulum (ER) (Olofsson *et al,* 1999). The VLDL secretion appears to be driven by the availability of plasma free fatty

acids (FFA) that act as fuel for lipid synthesis. Before being secreted in the blood the VLDL particle acquire other proteins and undergo massive remodelling by plasma lipid transfer proteins and lipases (Persson *et al*, 1989).

2.6.4 Lipase family

Lipase family consists of different enzymes (Aberg *et al*, 2003) which cause hydrolysis of glycerides present in different lipid fractions, resulting in formation of free fatty acid and different relatively smaller lipid fractions.

Lipoprotein lipase (LPL) is a protein secreted primarily by adipocytes and muscle cells into the circulation where it is bound to the luminal surface of capillary endothelial cells (Emmerich *et al*, 1992). In the presence of its cofactor, apo C-II (Abbate and Brunzell, 1990), lipoprotein lipase hydrolyzes triglycerides present in chylomicrons and very low density lipoproteins to free fatty acids (FFA) which can be utilized as sources of energy or re-esterified for storage. During the hydrolytic process LPL is intimately involved in the initial remodelling of the triglyceride-rich lipoprotein particles and thus plays a key role in both normal triglyceride and lipoprotein metabolism.

Hepatic lipase (HL) is synthesized in hepatocytes and transported to hepatic endothelial cells where it is bound by means of heparin sulphate (Thuren, 2000). The major role of HL appears to be hydrolysis of triglycerides and phosphoglycerides of HDL and IDL with the help of apo-C II and also LDL. It also acts in succession to lipoprotein lipase on TG rich lipoproteins catalysing the hydrolysis of intermediate density lipoproteins to produce chylomicron remnants and low density lipoproteins.

Hormone sensitive lipase (Kraemer and Shen, 2002) is an intracellular enzyme. It hydrolyses adipose tissue store of triglycerides which is the major source of FFA in the fasting state.

Endothelial lipase (EL) is synthesized by endothelial cells and is expressed in liver, lung, kidney and placenta, but not in skeletal muscle. It has a considerable molecular homology with lipoprotein lipase (LPL) and hepatic lipase (HL). As a lipase, EL has primarily phospholipase A1 activity, playing a role in HDL metabolism. However, recent study has shown that EL have role in metabolism of apo-B containing lipoproteins (Broedl *et al,* 2004).

2.6.5 Lipoprotein transport

There are two major pathways of lipoprotein transport namely the exogenous pathway and the endogenous pathway (Ginsberg, 1998) (figure 2.4). The exogenous pathway transports dietary lipids to the periphery and the liver and the endogenous pathway transports hepatic lipids to the periphery.

In the exogenous pathway (Ginsberg, 1998), cholesterol and fatty acids after intestinal absorption, are re-esterified to form triglyceride and cholesteryl ester (CE) in intestinal mucosal cells. These lipids are then packaged together with apo B_{48}, phospholipids, unesterified cholesterol, apo C-11 and apo E into nascent chylomicrons, secreted into the lacteals and transported via the thoracic duct into the blood. During transport in the circulation, chylomicrons are degraded to smaller chylomicron remnants which are recognised by the apo E receptors on hepatic parenchymal cells and rapidly removed from the blood stream (Ginsberg, 1990).

In the endogenous pathway (Ginsberg, 1998), triglycerides and cholesterol are transported to different tissues in the form of VLDL, IDl and LDL. The liver assmbles and secretes apo B-100 contatining lipoproteins, mainly VLDL. Fatty acids surplus in the liver are esterified to form triglyceride and are packed together with cholesterol, phospholipid, apolipoprotein B_{100}, apolipoprotein C-11 and apolipoprotein E and secreted into the blood as VLDL. During transport the VLDL undergoes delipidation and becomes smaller and denser resulting in the formation of IDL and later LDL. The LDL is removed from the circulation by LDL receptors present in the liver. The main structural protein that remains associated with VLDL, IDL and

LDL is apo B 100. Hence apoB-100 is a suitable marker for tracing the metabolic pathway of these lipoprotein particles (figure 2.5) .This is the fundamental principle employed in studies of the metabolism of apoB containing lipoproteins based on the use of stable isotopically labelled amino acid precursor (Duvillard *et al,* 2000).

Excess cholesterol from the periphery is transported back to the liver for excretion in the bile by a process called reverse cholesterol transport (figure 2.6). Both intestine and liver secrete individual apolipoproteins with a small amount of phospholipid. These proteins can absorb free cholesterol on the surface from different cell membranes, then becoming nascent HDL and are considered to be the initial step in reverse cholesterol transport. These particles can continue to absorb more free cholesterol and with the help of lecithin-cholesterol acyltransferase (LCAT) esterifies free cholesterol into cholesteryl esters (CE); forming mature HDLs that can be taken up by the liver via scavenger receptor class BI (SRB-1). Formation of CE increases the capacity of HDLs to accept more cholesterol and HDL transfer CE to other TG rich lipoproteins (VLDLs) with the help of cholesteryl ester transfer protein (CETP) resulting in formation of CE rich apo-B containing lipoproteins VLDL, IDL or LDL and TG rich HDL which then is further modified by hepatic lipase into smaller HDL and cleared from circulation (Brinton *et al*, 1991).

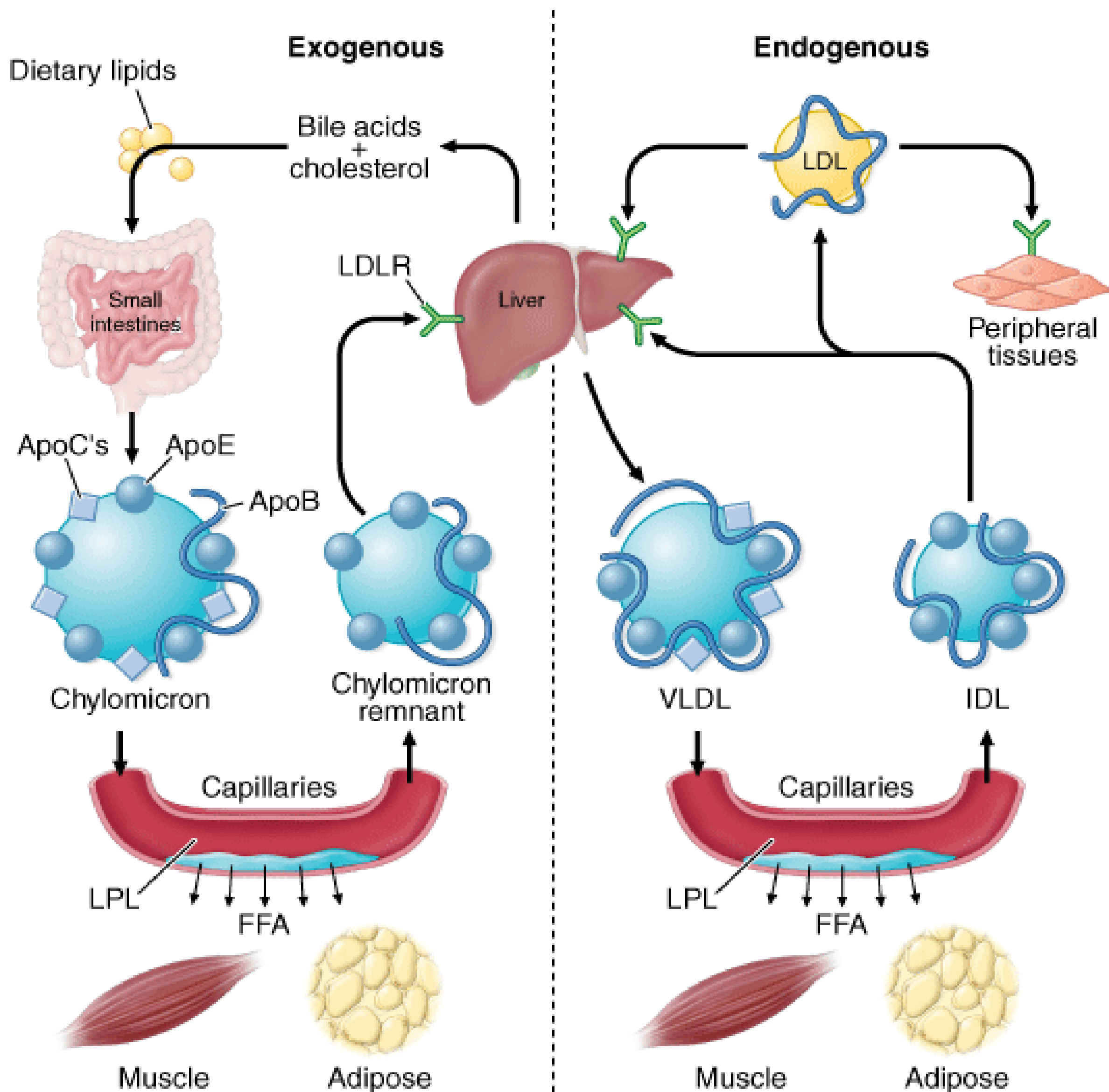


Figure 2.7: Diagram of the exogenous and endogenous lipoprotein metabolic pathways. (Adapted by Rader and Rader, 2008).

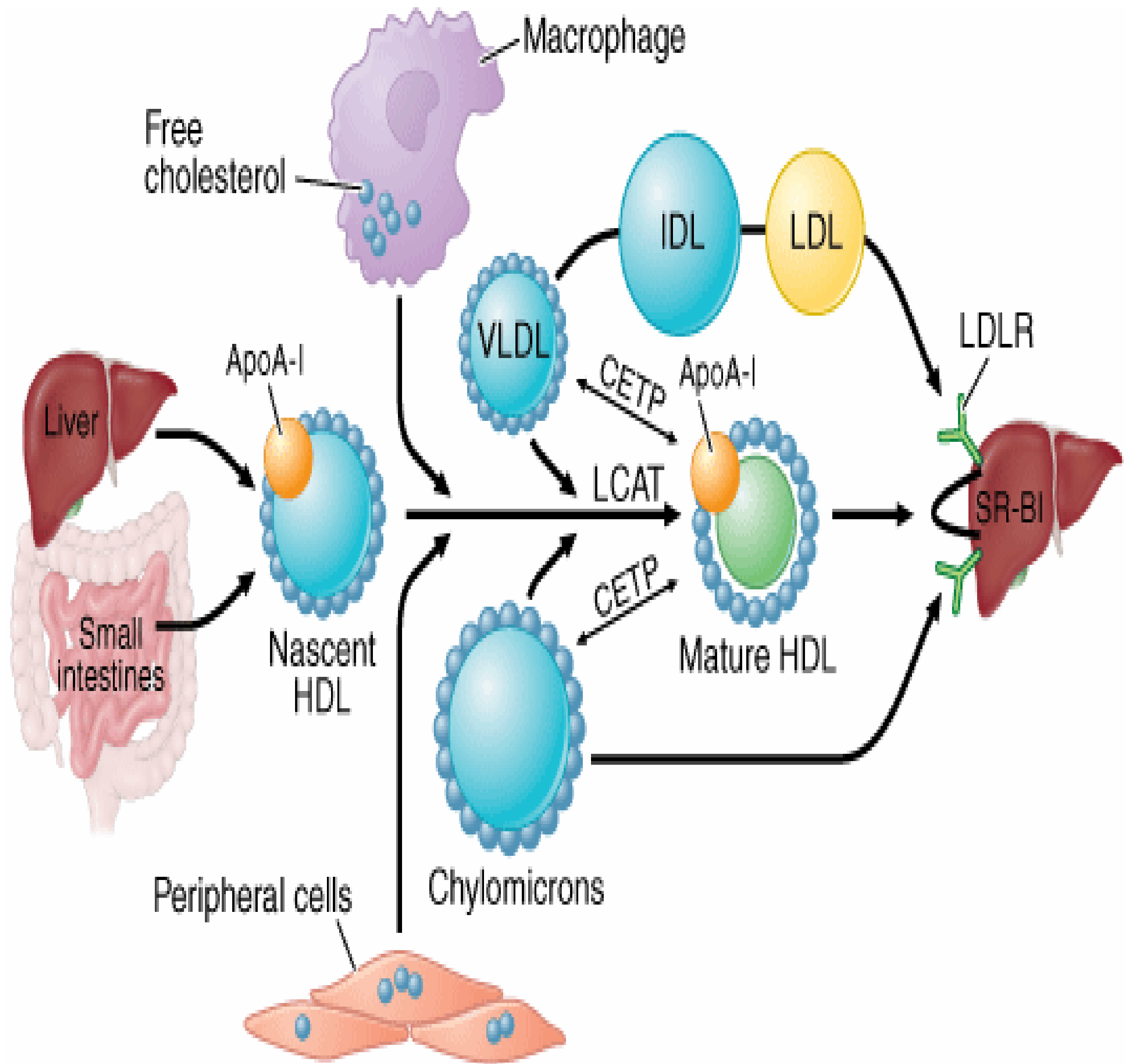


Figure 2.8: Diagram of reverse cholesterol transport. (Adapted by Rader and Rader, 2008)

2.6.6 Effect of HIV on fat distribution

The effect of HIV on fat distribution is also termed the mechanism of fat distribution or lipodystrophy in HIV infection. There are several pathogenic mechanisms and potential risk factors for fat redistribution (Lichenstein *et al*, 2003; Ion *et al*, 2006; Giralt *et al*, 2009). HIV patients are at higher risk of development of fat redistribution and the condition was first described as lipodystrophy syndrome by Carr *et al* (1998). Lipodystrophy syndrome is a common term used to describe several morphologic (fat atrophy and fat hypertrophy) and metabolic disturbances including dyslipidemia and insulin resistance found in patients with HIV infection. There are two major phenotypes described in HIV infected patients namely lipohypertrophy and lipoatrophy. Lipohypertrophy is characterised by the presence of fat accumulated in various parts of the body. The anatomical sites of fat deposits are abdominal, breast tissue and head-neck region (Nolan and Mallal, 2005). Lipoatrophy is characterised by peripheral fat wasting with loss of subcutaneous tissue in the face, arms, legs, and buttocks, producing an emaciated appearance (Lichenstein *et al*, 2005). Currently peripheral lipoatrophy is described as localized fat wasting in the face, arms, legs, and buttocks; Central lipohypertrophy, is described as fat accumulation in the abdomen, breasts, or dorsocervical region (buffalo hump) (Gazzard, 2008). Lipoatrophy should be distinguished from HIV wasting, which is a generalized loss of body fat and lean body mass.

Host factors: A number of host factors have been reported to cause fat abnormality in HIV infection (Lichenstein *et al*, 2003; 2007). These include older age (Miller *et al*, 2003), change in body mass index (Carter *et al,* 2006), duration of HIV-1 infection (Lichenstein *et al*, 2005), effectiveness of viral suppression, baseline degree of immunodeficiency and subsequent immune restoration with therapy, and white race (Kosmiski *et al*, 2001; Lichenstein *et al*, 2003; 2005). The risk of lipoatrophy has been found to be increased more in whites than in blacks (Lichenstein *et al*, 2003). Gender-based differences in presentation have been reported. Women are more likely to report fat accumulation in the abdomen and breasts, whereas men are more likely to describe fat depletion from the face and extremities (Miller *et*

al, 2003). Older age is a consistently reported risk factor; body fat changes occur naturally with aging and body fat distribution abnormalities have also been reported in HIV-infected children (Alves *et al*, 2008).

Antiretroviral therapy: This is an example of risk factors for fat distribution in HIV. The type and duration of antiretroviral drug exposure have been associated with fat distribution abnormalities. Patients starting antiretroviral treatment for the first time may be presented with initial increases in limb fat during the first few months of therapy, followed by a progressive decline during the ensuing years. In contrast, truncal fat increases initially and then remains stable during the ensuing years, resulting in relative central adiposity. Changes in limb and central fat masses may become clinically evident in 20 to 35 percent of patients after about 12 to 24 months of combination antiretroviral therapy (Mallon *et al*, 2003). Fat accumulation or lipohypertrophy is more common with PI use and fat loss or lipoatrophy with thymidine analogues. PI has been shown to contribute to the central fat accumulation (Nolan and Mallal, 2004; Lichenstein *et al*, 2005). Longer treatment with PI- containing HAART regimens is reported to result in reduced lipoprotein lipase activity (Yarasheski *et al,* 2003).

Impairment of adipocyte function: HIV infection has an effect on adipocyte function. Adipocytes are fat cells that produce multiple bioactive peptides termed adipokines or adipocytokines that participate in lipid metabolism, immunity, blood pressure, insulin sensitivity, appetite and energy balance (Ahima and Flier, 2000). Adiponectin is expressed in white adipose tissue (Diez and Iglesias, 2003), it sensitizes peripheral tissue to insulin (Goldfine and Kahn, 2003) and has anti-atherogenic properties by its inhibition of monocyte adhesion to endothelial cells and macrophage transformation to foam cells (Ouchi *et al,* 1999; 2000). High plasma adiponectin was associated with reduction of risk of myocardial infarction in men (Cavusoglu *et al*, 2006). Low plasma circulating levels of adiponectin has been observed in HIV subjects on stavudine (Podzanczer *et al*, 2007).

HIV infection plays a role in adipocyte apoptosis. Lipoatrophic tissues from HIV subjects were demonstrated to show increased expression of Tissue Necrosis Factor (TNF) – a cytokine known to induce apoptosis of adipocytes. A genetic case-control study (Maher *et al*, 2002) conducted in HIV-positive patients both with and without lipodystrophy found a significant difference between groups in the frequency of polymorphism -238 in the region of the TNF-gene, suggesting that the -238 polymorphism is a determinant in the development of HIV-related lipodystrophy. Nolan *et al* (2003) using fat biopsy samples from HIV patients showed increased expression of several adipocyte specific pro- inflammatory cytokines (IL-18, 1L-6, TNF, IL-8, IL-12) and significant correlation with increased lipoatrophy.

HIV infection also plays a role in adipocyte differentiation. Abnormalities of fat distribution in individuals with HIV infection has been linked to immune or inflammatory factor. Pro-inflammatory cytokines are known to stimulate lipolysis and inhibit adipose tissue lipogenesis (Johnson *et al*, 2004; Chaparro *et al*, 2005; De brenzo *et al,* 2008). Bastard *et al* (2002) showed that adipocytes from patients with lipoatrophy when treated with NRTIs and PIs have higher levels of sterol-regulatory element-binding-protein-1 (SREBP1c) an adipocyte transcription factor and higher expression of TNF-, but the adipocytes were smaller and tended to cluster, suggesting an impairment of differentiation despite the increased amount of SREBP1c protein.

Mitochodrial toxicity: Antiretroviral therapy has been implicated to cause mitochodrial toxicity: In the normal situation, mitochondrial DNA (mtDNA) encodes for proteins in the respiratory chain, situated at the inner mitochondrial membrane. Most respiratory enzymes are encoded by nuclear DNA (nDNA). Replication of mtDNA is regulated by the enzyme DNA polymerase. But in inherited mitochondrial diseases, parts or whole of mtDNA have been mutated, results in altered mtDNA-encoded protein leading to mitochondrial dysfunction. Inhibition of mitochondrial DNA polymerase is associated with nucleoside analogues and has been hypothesized to play a role in fat redistribution. During NRTI treatment, DNA polymerase will be inhibited, leading to mitochondrial depletion (Brinkman

et al, 1999). Mitochondrial DNA depletion has been shown to correlate poorly with respiratory chain activity (Stankov, 2007).

Genetic predisposition: Mitochondrial haplotypes which is defined by pattern of single nucleotide polymorphism in mitochondrial genes and mutation in haemochromatosis gene (HFE) may have effect on fat distribution (Hulgan *et al,* 2008). HIV-infected patients with a common mutation in the hemochromatosis gene (HFE), in combination with mitochondrial DNA haplogroup J, may be protected from the development of lipoatrophy associated with antiretroviral therapy. The relationship between HFE gene variants, mitochondrial haplogroups, and peripheral lipoatrophy during ART was studied (Hulgan *et al,* 2008). It was reported that patients with the HFE 187C >G polymorphism were less quick to develop lipoatrophy during ART. This association was independent of other factors including age, CD4 lymphocyte count, and specific antiretroviral therapy received Hulgan *et al,* 2008). In contrast TNF-gene -238G/A promoter polymorphism were associated with a more rapid onset of lipodystrophy (Nolan *et al,* 2008) and in the ACTG 5202 study, patients with mitochondrial haplotype-I were found to have a tendency to more periphral fat loss compared to non-I haplotypes.

Insulin Resistance and Diabetes: Before the use of highly active antiretroviral therapy, blood glucose abnormalities were infrequently seen in people with HIV. But soon after protease inhibitors came into widespread, the U.S. Food and Drug Administration (FDA) issued a health advisory warning of an association between PIs and hyperglycaemias and diabetes mellitus (FDA, 1997). Since then, there have been continued reports of insulin resistance and diabetes in people using HAART especially with patients taking PIs (Walli *et al*, 1998; Tsiodras *et al,* 2000).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study design and subjects recruitment

This is a prospective study on the effect of HIV with or without malaria co-infection and antiretroviral therapy on some cardiac markers and Apolipoprotein profile of both Adult male and female subjects.

This study was conducted at Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi in Anambra State. Based on 3.1 % prevalence rate of HIV in Nigeria (NACA, 2011) and using the formular of Naing *et al*, (2006) for sample size calculation, a total of 400 (M=220, F= 180) Adult subjects aged between 18 and 60 (38± 9) years participated in this study. They were randomly recruited by convenience sampling from the Voluntary and Counseling Unit (VCT) and Antiretroviral Unit (ART) of NAUTH between September, 2012 and January, 2013 and symptomatic HIV subjects without ART that later commenced therapy were followed up between November to August, 2014. They all underwent HIV and *Plasmodium falciparum* screening. The serum samples were screened for HIV infection using two Immunochromatographic techniques respectively. Similarly, the blood samples were screened for malaria parasite infection using Giemsa stained thick and thin blood films for microscopic detection of malaria parasite and rapid chromatographic immunoassay for qualitative detection of circulating Plasmodium falciparum antigen in the whole blood. Using the World Health Organization (WHO, 2006), staging for HIV as a guide, the subjects were grouped as follows:

Symptomatic HIV on Antiretroviral therapy (ART) (n = 100, M = 50, F = 50) of these, 50 were symptomatic HIV on ART with malaria co-infection.

Symptomatic HIV not on ART (n = 100, M = 64, F = 36) of these, 50 were symptomatic HIV not on ART with malaria co-infection. Thirty (n=30; male=13; female= 17) of the symptomatic HIV subjects not on ART without malaria co-infection were followed up on

commencement of ART for 12 months. Their blood samples were drawn at 3, 6, 9 and 12 months respectively and study parameters analysed. Parameters were re-classified and analysed, based on 1.5 ng/mL Troponin diagnostic cut off for myocardial infarction (NCCLS, 1997).

Asymptomatic HIV subjects (n = 100, M = 57, F = 43) of these, 50 were asymptomatic HIV with malaria co-infection.

HIV seronegative control subjects (n = 100, M = 49, F = 51) of these, 50 were malaria positive.

3.2 Blood sample collection

Ten milliliters (10 ml) of fasting blood samples were collected from all the subjects in this study. Two milliliters (2 ml) of blood samples were collected into EDTA sample containers for malaria antigen estimation, thick and thin film for malaria microscopy and CD4 counts. The remaining eight milliliter (8 ml) of blood sample were collected into plain tubes and allowed to clot, centrifuged, separated and aspirated into plain sample tubes and kept frozen until assay for apo A1, A2, B, C2, C3 and E; lipid profile, for HIV detection, troponin I, CK-total, CK-MB, myoglobin, lactate dehydrogenase and aspartate dehydrogenase.

3.3 Inclusion and exclusion criteria

Subjects adjudged as clinically asymptomatic HIV (stage 1) positives with or without malaria infection; clinically symptomatic HIV (stage 2) positives not on ART with or without malaria infection and clinically symptomatic HIV (stage 2) positives on ART with or without malaria infection were included in this study. Individuals presenting with HIV stage 3 and 4 (see WHO HIV classification) were excluded from the study. Subjects presented with history of smoking, hypertension, tuberculosis, diabetes, heart, renal diseases and any other clinical condition apart from HIV and malaria falciparum infection were excluded from the study.

3.4 Questionaire

Questionnaires were included to get the biodata of the participants and other health information that were helpful in the interpretation and analysis of results, in this study.

3.5 Quality control measures

Quality control sera were analysed along test in each batch of analysis these were compared with the reference values of the control sera. Standard deviation and coefficient of variation were calculated on them.

3.6 Anti-Retroviral drug used:

The three antiretroviral drugs used for treatment in these HIV subjects were based on WHO recommendation (WHO, 2003). The combined therapy was made up of two Nucleoside reverse transcriptase inhibitors (zidovudine and lamivudine) and one Non- nucleoside reverse transcriptase inhibitor (nevirapine). Zidovudine, lamivudine and nevirapine were taking together as combined therapy at the same time through an oral route as prescribed below. These drugs are administered on the subjects to improve their CD4 counts to normal value as in the general population (WHO, 2003):

Zidovudine (ZDV) or Azitrothymidine (AZT) at a dosage of 300mg twice was given daily at an interval of 8 hours.

Lamivudine (3TC, Epivir) at a dosage of 150mg twice was given daily at an interval of 8 hours.

Nevirapine (NUP, Viramine) at a dosage of 200mg twice was given daily at an interval of 8 hours.

Site for analysis.

The laboratory analyses were carried out in the HIV unit, in Chemical Pathology laboratory, both in Nnamdi Azikiwe University Teaching Hospital and Human Biochemistry Laboratory, Nnamdi Azikiwe University Nnewi, Anambra State.

3.8 Calculation of sample size:

This was done using the formular of Naing *et al*, (2006) $N = z^2 p\ q / d^2$

Where q = 1-p, d= desired levels of significance (0.05), p = prevalence rate in % (3.1%) (NACA, 2011).

N= minimum sample size, z = confidence interval of 95% equivalent to confidence coefficient of 1.96 of normal distribution

$N = 1.96^2 \times 0.031\ (1-0.031) / 0.05^2 = 48$. The minimum sample size = 48

3.9 Ethical approval

The study design was approved by the Nnamdi Azikiwe University Teaching Hospital Board of ethics Committee and the participants gave both written and oral informed consents.

3.10 Methods of Assay

3.10.1 Determination of Antibodies to HIV-1 and HIV-2 in Human plasma.

Principle

The test was based on the agglutination principle. Agglutination occurs when serum containing HIV-1 and 2 antigens were tested against their corresponding antibodies. The complex formed was observed in the presence of colour incorporated to aid visual examination.

Procedure

Two different methods were used, namely, Abbott determine TM HIV -1 and HIV-2 kit, which is an in-vitro visually read immunoassay (Abbott Japan Co.Ltd.Tokyo, Japan) and HIV-1 and 2 STAT-PAK Assay kit, which is an Immunochromatographic test for the quantitative detection of antibodies to HIV-1 and HIV-2 in Human plasma (CHEMBIO Diagnostic system, Inc, New York, USA). For the Abbott determine TM HIV -1 and HIV-2 kit, the procedure described by the manufacturer (Abbott Japan Co.Ltd.Tokyo, Japan) was used for the analysis. Briefly, 50 µl of participant serum samples separated from the

corresponding whole blood samples in EDTA were applied to the appropriately labeled sample pad. After 15 minutes but not more than 60 minutes of sample application, the result was read. This method has inherent quality control that validates the results. For the Immunochromatographic method for HIV -1 and HIV-2, the procedure described by the manufacturer was used for the analysis. In brief, a drop of participant's plasma was dispensed into the sample well in the appropriately labeled sample pad. Three drops of the buffer supplied by the manufacturer was added into the appropriately labeled sample pad. The results of the test were read at 10 minutes after the addition of the running buffer. This method has inherent quality control and validates the results.

3.10.2 Determination of $CD4^{+}$T cells counts by CyFlows SL-Green

Principle

In a flow cytometry, the cells were illuminated with a laser beam. The amount of a laser light that was scattered off the cells as they passed through the laser beam was measured, which gives information concerning the size of the cells.

Procedure

50 µl of EDTA whole blood was collected into PARTEC test tubes (Rohren tube). Then 10 µl of $CD4^{+}$ T antibody was added into the tube. The contents was mixed and incubated in the dark for15 minutes at room temperature. 800 ml of CD4 buffer was added into the mixture and mixed gently. Then the Partec tube was plugged on the Cyflow counter and the $CD4^{+}$ T cells were displayed as peaks and interpreted as figures.

3.10.3 Detection of Plasmodium falciparum parasite

(a) Using Plasmodium falciparum malaria antigen rapid test device.

Principle

The principle of Plasmodium falciparum antigen detection was based on a rapid chromatographic immunoassay for the qualitative detection of circulating *Plasmodium falciparum* antigen in the whole blood. This method utilizes gold conjugate to selectively detect plasmodium antigen.

Procedure

The procedure was as described by the manufacturer (Access Bio, Incorporated, New Jersey, USA). 5 µl of whole blood was added into sample well of the test device and 60 µl of assay buffer was added also into the sample well. The analysis was read within 20 minutes. The presence of two colour bands indicate a positive result but the presence of one colour band indicates a negative result.

(b) Determination of malaria parasitaemia by Thick and thin film as described by WHO (1995).

Principle

The principle of malaria parasite density was based on the examination of stained film using the x 100 oil immersion objective. 200 and 500 leucocytes were counted in a field and at the same time, the number of malaria parasite and density present in the field were counted.

Procedure

Thick and thin films will be prepared for each participant's blood sample. The thin films were fixed with methanol and both thick and thin films were stained with Giemsa (1 in10 dilution) for 10 minutes, after which they were examined microscopically with oil immersion (x 100) objective. The malaria parasite counting was done using the thick blood films while the thin blood films were used for species identification. Malaria parasites were counted according to the method of World Health Organisation (1995). 200 leukocytes were counted and if 10 or more parasites were identified, then the number of parasites per 200 leucocytes was recorded; but if after counting 200 leukocytes and 9 or less parasites identified then, 500 leukocytes was recorded. In each case the parasite count in relation to the leukocyte count was converted to parasite per microlitre of blood using this mathematical formular: Malaria parasite density /µl= number of parasites x 8000 Number of leukocytes Where 8000, is the average number of leukocyte per microlitre of blood, which is taken as the standard (WHO, 1995).

3.10.4 Quantitative determination of Apolipoprotein A_1 in human sera as described by Tiez (1983).

Principle

Turbidimetric test was used for the measurement of apolipoprotein A_1. Anti- Apo A_1 antibodies were mixed with samples containing Apo A_1, form insoluble complexes. These complexes cause an absorbance change, dependent upon the Apo A_1 concentration of the patient's sample, which was quantitated by dividing the absorbance of sample by that of the calibrator and multiply by the concentration of the calibrator.

Procedure

The procedure was as described by the manufacturer (Spinreact laboratories limited, Spain).

The spectrophotometer was zeroed with distilled water. The reacting mixture was carried out at 37 ^{O}C. 800 µ/l of Reagent R1 (Tris buffer, 20 mmol/L. PEG, pH 8.3, sodium azide 0.95 g/L) was dispensed into a cuvette and 7 µ/l of calibrator was added respectively, mixed and the absorbance (A_1) of calibrator was read at 340 nm. The sample was treated the same way as the calibrator and its absorbance reading as A_1 sample. Immediately, 200 µ/l of Reagent R2 (Anti-human apolipoprotein A-1 goat- polyclonal antibody, tris buffer, 50 mmol/L, pH 7.5, sodium azide 0.95 g/L) was dispensed into each of the same cuvette, mixed and was read again at 340 nm after 2 minutes as A_2 for calibrator and sample respectively.

Calculations

$$\frac{(A2-A1)\ \text{sample}}{(A2-A1)\ \text{calibrator}} \times \text{Calibrator concentration} = \text{mg/dl Apo } A_1$$

3.10.5 Quantitative determination of Apolipoprotein A_2 in human sera as described by Tiez (1983).

Principle

Turbidimetric test was used for the measurement of apolipoprotein A_2. Anti- Apo A_2antibodies were mixed with samples containing Apo A_2, form insoluble complexes. These complexes cause an absorbance change, dependent upon the Apo A_2 concentration of the

patient sample, which was quantitated by dividing the absorbance of sample by that of the calibrator and multiply by the concentration of the calibrator.

Procedure

The procedure was as described by the manufacturer (Spinreact laboratories limited, Spain). The spectrophotometer was zeroed with distilled water. The reacting mixture was carried out at 37 ^{O}C. 300 µ/l of Reagent R1 (2-amino-2-hydroxymethyl-1, 3-propanediol buffer, 100 mmol/L, pH 8.5, macrogol) was dispensed into a cuvette and 5 µ/l of calibrator was added respectively, mixed and the absorbance (A1) of calibrator was read at 600 nm. The sample was treated the same way as the calibrator and its absorbance reading as A1 sample. Immediately, 100 µ/l of Reagent R2 (Anti-human apolipoprotein A_2 goat- polyclonal antibody) was dispensed into each of the same cuvette, mixed and was read again at 600 nm after 5 minutes as A2 for calibrator and sample respectively.

Calculations

$$\frac{(A2-A1)\ \text{sample}}{(A2-A1)\ \text{calibrator}} \times \text{Calibrator concentration} = \text{mg/dl Apo } A_2$$

3.10.6 Quantitative determination of Apolipoprotein B in human sera as described by Tiez (1983).

Principle

Turbidimetric test was used for the measurement of apolipoprotein B. Anti- Apo B antibodies were mixed with samples containing Apo B, form insoluble complexes. These complexes cause an absorbance change, dependent upon the Apo B concentration of the patient sample, which was quantitated by dividing the absorbance of sample by that of the calibrator and multiply by the concentration of the calibrator.

Procedure

The procedure was as described by the manufacturer (Spinreact laboratories limited, Spain).

The spectrophotometer was zeroed with distilled water. The reacting mixture was carried out at 37 ^{O}C. 800 µ/l of Reagent R1 (Tris buffer, 20 mmol/L, PEG, pH 8.3, sodium azide, 0.95

g/L) was dispensed into a cuvette and 7 µ/l of calibrator was added respectively, mixed and the absorbance (A1) of calibrator was read at 340 nm. The sample was treated the same way as the calibrator and its absorbance reading as A1 sample. Immediately, 200 µ/l of Reagent R2 (Anti-human apolipoprotein B goat- polyclonal antibody, Tris buffer, 50 mmol/L, pH 7.5, sodium azide, 0.95 g/L) was dispensed into each of the same cuvette, mixed and was read again at 340 nm after 2 minutes as A2 for calibrator and sample respectively.

Calculations

$$\frac{(A2-A1)\ \text{sample}}{(A2-A1)\ \text{calibrator}} \times \text{Calibrator concentration} = \text{mg/dl Apo B}$$

3.10.7 Quantitative determination of Apolipoprotein C_2 in human sera as described by Tiez (1983).

Principle

Turbidimetric test was used for the measurement of apolipoprotein C_2. Anti- Apo C_2 antibodies were mixed with samples containing Apo C_2, form insoluble complexes. These complexes cause an absorbance change, dependent upon the Apo C_2 concentration of the patient sample, which was quantitated by dividing the absorbance of sample by that of the calibrator and multiply by the concentration of the calibrator.

Procedure

The procedure was as described by the manufacturer (Spinreact laboratories limited,Spain).

The spectrophotometer was zeroed with distilled water. The reacting mixture was carried out at 37 ^{O}C. 750 µ/l of Reagent R1 (Tris buffer, 100 mmol/L, PEG 4000, pH 8.5, sodium azide, 0.95 g/L) was dispensed into a cuvette and 25 µ/l of calibrator was added respectively, mixed and the absorbance (A_1) of calibrator was read at 340 nm. The sample was treated the same way as the calibrator and its absorbance reading as A_1 sample. Immediately, 250 µ/l of Reagent R2 (Anti-human apolipoprotein C-11 goat- polyclonal antibody, Tris buffer, 100 mmol/L, pH 7.2, sodium azide, 0.95 g/L) was dispensed into each of the same cuvette,

mixed and was read again at 340 nm after 5 minutes as A_2 for calibrator and sample respectively.

Calculations

(A2-A1) sample x Calibrator concentration = mg/dl Apo C_2

(A2-A1) calibrator

3.10.8 Quantitative determination of Apolipoprotein C_3 in human sera as described by Tiez (1983).

Principle

Turbidimetric test was used for the measurement of apolipoprotein C_3. Anti- Apo C_3antibodies were mixed with samples containing Apo C_3, form insoluble complexes. These complexes cause an absorbance change, dependent upon the Apo C_3 concentration of the patient sample, which was quantitated by dividing the absorbance of sample by that of the calibrator and multiply by the concentration of the calibrator.

Procedure

The procedure was as described by the manufacturer (Spinreact laboratories limited, Spain).

The spectrophotometer was zeroed with distilled water. The reacting mixture was carried out at 37 ^{O}C. 750 µ/l of Reagent R1 (Tris buffer, 100 mmol/L, PEG 4000, pH 8.5, sodium azide, 0.95 g/L) was dispensed into a cuvette and 20 µ/l of calibrator was added respectively, mixed and the absorbance (A1) of calibrator was read at 340 nm. The sample was treated the same way as the calibrator and its absorbance reading as A_1 sample. Immediately, 250 µ/l of Reagent R2 (Anti-human apolipoprotein C_3 goat- polyclonal antibody, Tris buffer, 100 mmol/L, pH 7.2, sodium azide, 0.95 g/L) was dispensed into each of the same cuvette, mixed and was read again at 340 nm after 5 minutes as A2 for calibrator and sample respectively.

Calculations

(A2-A1) sample x Calibrator concentration = mg/dl Apo C_3

(A2-A1) calibrator

3.10.9 Quantitative determination of Apolipoprotein E in human sera as described by Tiez (1983).

Principle

Turbidimetric test was used for the measurement of apolipoprotein E. Anti- Apo E antibodies were mixed with samples containing Apo E, form insoluble complexes. These complexes cause an absorbance change, dependent upon the Apo E concentration of the patient sample, which was quantitated by dividing the absorbance of sample by that of the calibrator and multiply by the concentration of the calibrator.

Procedure

The procedure was as described by the manufacturer (Spinreact laboratories limited,Spain).

The spectrophotometer was zeroed with distilled water. The reacting mixture was carried out at 37 ^{O}C. 750 µ/l of Reagent R1 (Tris buffer, 100 mmol/L, PEG 4000, pH 8.5, sodium azide, 0.95 g/L) as dispensed into a cuvette and 25 µ/l of calibrator was added respectively, mixed and the absorbance (A1) of calibrator was read at 340 nm. The sample was treated the same way as the calibrator and its absorbance reading as A1 sample. Immediately, 250 µ/l of Reagent R2 (Anti-human apolipoprotein E goat- polyclonal antibody, Tris buffer, 100 mmol/L, pH 7.2, sodium azide, 0.95 g/L) was dispensed into each of the same cuvette, mixed and was read again at 340 nm after 5 minutes as A2 for calibrator and sample respectively.

Calculations

$$\frac{(A2-A1)\ \text{sample}}{(A2-A1)\ \text{calibrator}} \times \text{Calibrator concentration} = \text{mg/dl Apo E}$$

3.10.10 Quantitative determination of Troponin I (cTnI) in human sera as described by Tiez (1995).

Principle

The principle of the test was based on sandwiched enzyme-linked immunosorbent assay (ELISA). In this technique, three mouse monoclonal anti-troponin antibodies were

immobilized on a microtitre well. A sample containing the Troponin 1 antigen was added and allowed to react with the bound antibodies. A fourth antibody linked with enzyme conjugate was added and allowed to react with d bound antigens. The well was washed to remove free antigens and antibodies. Enzyme substrate was added and the colour reaction product formed was measured. The concentration of troponin 1 was directly proportional to the colour intensity of the test sample measured.

Standard cTn1 Calibration Curve.

Calibration curve was prepared by the method as described by the manufacturer (Life Sciences Advanced Technologies, Inc, USA). The following concentrations of standards of cTn1 were provided: 0 ng/ml, 2 ng/ml, 7.5 ng/ml, 30 ng/ml and 75 ng/ml. Each standard was treated in the same procedure as described for the test sample. A standard calibrate curve was prepared by plotting concentrations (ng/ml) of these standards against their absorbances.

Procedure

The procedure was as described by the manufacturer (Life Sciences Advanced Technologies, Inc, USA). 100 µl of standards, specimens and controls were dispensed into appropriates microtiter wells. 100 µl enzyme conjugate reagent was dispensed into each well, gently mixed for 30 seconds and incubated at room temperature for 90 minutes. The incubation mixture was emptied into a sink, rinsed 5 times with deionized water and the residual water droplet absorbed with absorbent paper. Then, 100 µl of tetramethylbenzidine (TMB) reagent was added into each well, gently mixed for 10 secs and allowed to developed colour at room temperature for 20 minutes. The reaction was stopped by the addition of 100 µl hydrochloric acid into each well and gently mixed for 30 seconds.

The absorbance of the standard and test were read within 15 minutes of performing the analysis, after zeroing the machine with blank at 450nm wavelength using spectrophotometer. The concentrations of cTn1 in specimens were extrapolated from the graph.

3.10.11 Quantitative determination of Myoglobin in human sera as described by Tiez (1995).

Principle

The principle of the test was based on sandwiched enzyme-linked immunosorbent assay (ELISA). In this technique, myoglobin antibody was immobilized on a microtitre well. A sample containing the myoglobin antigen was added and allowed to react with the bound antibodies. A fourth antibody linked with enzyme conjugate was added and allowed to react with the bound antigen. The well was washed to remove free antigen and antibody. Enzyme substrate was added and the colour reaction product formed was measured. The concentration of myoglobin was directly proportional to the colour intensity of the test sample.

Standard myoglobin Calibration Curve.

Calibration curve was prepared by the method as described by the manufacturer (Life Sciences Advanced Technologies, Inc, USA). The following concentrations of standards of myoglobin were provided and they were prediluted 10 fold: 0 ng/ml, 25 ng/ml, 100 ng/ml, 250 ng/ml, 500 n/g and 1000 ng/ml. Each standard was treated in the same procedure as described for the test sample. A standard calibrate curve was prepared by plotting concentrations (ng/ml) of these standards against their absorbances.

Procedure

The procedure was as described by the manufacturer (Life Sciences Advanced Technologies, Inc, USA). 20 µl of already 10 fold diluted standards, 10 fold diluted specimen and 10 fold diluted controls was dispensed into appropriates microtiter wells. 200 µl enzyme conjugate reagent was dispensed into each well, gently mixed for 30 seconds and incubated at room temperature for 45 minutes. The incubation mixture was emptied into a sink, rinsed 5 times with deionized water and the residual water droplet absorbed with absorbent paper. Then, 100 µl of tetramethylbenzidine (TMB) reagent was added into each well, gently mixed for 5 seconds and allowed to developed colour at room temperature for 20 minutes. The reaction

was stopped by the addition of 100 µl hydrochloric acid into each well and gently mixed for 30 seconds.

The absorbance of the standard and test were read within 15 minutes after zeroing the machine with blank at 450 nm wavelength using spectrophotometer. The concentrations of myoglobin in specimens were extrapolated from the graph.

3.10.12 Quantitative determination of total Creatine kinase (CK) in Human sera as described by Tiez (1983).

Principle

CK was determined kinetically by catalyzes the reversible interconversion of creatine phosphate to creatine.

Procedure

The procedure was as described by the manufacturer (Agappe Diagnostics, Switzerland). 100 µl of standards, specimens and controls were dispensed into appropriates test-tubes. 1000 µl of working reagent (D-glucose 125 mmol/L, N-Acetyl-L-cysteine 25 mmol/L, magnesium acetate, 12.5 mmol/L, NADP 2.4 mmol/L, EDTA 2 mmol/L, Hexokinase > 6800 U/L, creatine phosphate 250 mmol/L, ADP 15.2 mmol/L, AMP, 25 mmol/L, Diadenosine pentaphosphate 103 mmol/L, G-6-PDH > 8800 U/L) were dispensed into each test-tube respectively, gently mixed and incubated at 37 ^{o}C for 1 minute. The change in absorbance per minute was measured within 3 minutes at 340 nm.

Calculation:

Creatine kinase Activity (U/L) = (▲OD/ 3 minutes) x 4127

3.10.13 Quantitative determination of Creatine kinase (CK)- MB in Human sera as described by Tiez (1983).

Principle

The principle involves measurement of creatine kinase activity in the presence of an antibody to creatine kinase- M monomer. This antibody completely inhibits the activity of CK-MM

and half of the activity of CK-MB, while not affecting the B subunit activity of CK-MB and CK-BB. Then we use CK method to quantitatively determine CK-B activity. The CK-MB activity was obtained by multiplying the CK-B activity by two.

Procedure

The procedure was as described by the manufacturer (Agappe Diagnostics, Switzerland). 40 µl of standards, specimens and controls were dispensed into appropriates test-tubes. 1000 µl of working reagent (imidazole (pH 6.7), 125 mmol/L, D-glucose 25 mmol/L, N-Acetyl-L-cysteine 25 mmol/L, magnesium acetate, 12.5 mmol/L, NADP 2.52 mmol/L, EDTA 2.02 mmol/L, Hexokinase > 6800 U/L, creatine phosphate 250 mmol/L, ADP 15.2 mmol/L, AMP, 25 mmol/L, Diadenosine pentaphosphate 103 mmol/L, G-6-PDH > 8800 U/L) were dispensed into each test-tube respectively, gently mixed and incubated at 37 ^{o}C for 100 seconds. The change in absorbance per minute was measured within 3 minutes at 340 nm.

Calculation:

Creatine kinase –MB Activity (U/L) = (▲OD/ 3 minute) x 8254.

3.10.14 Quantitative determination of Aspartate amino transferase (AST) as will be described by Tiez (1983).

Principle

AST was determined kinetically by catalyzes the reversible interconversion of aspartate to glutamate.

Procedure

The procedure was as described by the manufacturer (Agappe Diagnostics, Switzerland). 100 µl of standards, specimens and controls were dispensed into appropriates test-tubes. 1000 µl of working reagent (Tris buffer, 88 mmol/L, L-aspartate, 260 mmol/L, MDH, > 600 U/L, LDH > 900 U/L, NADH, 0.20 mmol/l, α- ketoglutarate 12 mmol/L)) were dispensed into each test-tube respectively, gently mixed and incubated at 37 ^{o}C for 100 seconds. The change in absorbance per minute was measured within 3 minutes at 340 nm.

Calculation:

AST Activity (U/L) = (▲OD/ 3 minutes) x 1768

3.10.15 Quantitative determination serum lactate dehydrogenase as described by Tiez (1983).

Principle

LDH was determined kinetically by catalyzes the reversible interconversion of lactate to pyruvate.

Procedure

The procedure was as described by the manufacturer (Agappe Diagnostics, Switzerland). 10 µl of standards, specimens and controls were dispensed into appropriates test-tubes. 1000 µl of working reagent (Tris buffer, 80 mmol/L, pyruvate 1.6 mmol/L, sodium chloride, 200 mmol/L NADH, 240 mmol/L) were dispensed into each test-tube respectively, gently mixed and incubated at 37 ^{o}C for 1 minute. The change in absorbance per minute was measured within 3 minutes at 340 nm.

Calculation:

LDH Activity (U/L) = (▲OD/ 3 minutes) x 16030

3.10.16 Quantitative determination of serum Cholesterol as described by Allain *et al* (1974).

Principle

Cholesterol is present in serum as cholesterol ester and free cholesterol. In the serum, the cholesterol esters are hydrolysed by cholesterol esterase to total cholesterol, which is measured by oxidizing with cholesterol oxidase to form hydrogen peroxide. The hydrogen peroxide in turn reacts with phenol and 4-aminoantipyrine to form red quinonemine dye. The intensity of the dye formed is directly proportional to the concentration of cholesterol present in the sample.

Procedure

The procedure was as described by the manufacturer (Randox Laboratories, UK). 10 µl of standards, specimens and controls were dispensed into appropriately labeled standards, specimens and controls tubes respectively. 1 ml of cholesterol reagent containing (phenol- 6 mmol/L, pipes buffer- 50 mmol/L, 4-amino antipyrine- 0.3 mmol/L, cholesterol oxidase- 100 U/L, cholesterol estrase- 150 U/L and peroxidase 800 U/L) was added to each of the tube. The reagent blank was prepared similarly with the use of 10 µl of distilled water. These were incubated for 10 minutes at room temperation and absorbance measured at 546 nm against reagent blank and the concentration of serum cholesterol was calculated.

3.10.17 Quantitative determination of serum Triglyceride as described by Buccolo and David (1973).

Principle

Triglyceride in the serum is hydrolysed by lipoprotein lipase to glycerol and fatty acid. Glycerol oxidase ii turn oxidizes glycerol to glycerol-3-phosphate which is further oxidizes to hydrogen peroxide. The hydrogen peroxide in turn reacts with phenol and 4-aminoantipyrine and 4-chlorophenol to form a red coloured complex. The intensity of the dye formed is directly proportional to the concentration of triglyceride present in the sample.

Procedure

The procedure was as described by the manufacturer (Randox Laboratories, UK). 10 µl of standards, specimens and controls were dispensed into appropriately labeled standards, specimens and controls tubes respectively. 1000 µl of enzyme reagent was added to each of the tube. The reagent blank was prepared similarly with the use of 10 µl of distilled water. These were incubated for 10 minutes at room temperation and their absorbances measured at 546 nm against reagent blank and the concentration of serum cholesterol was calculated.

3.10.18 Quantitative determination of serum High Density Lipoprotein Cholesterol (HDL-C) as described by Assmann *et al* (1983).

Principle

Low Density Lipoprotein is precipitated by the addition of phosphotungstic acid in the presence of magnesium ions. The HDL fraction remains in the supernatant and this is determined by cholesterol assay.

Procedure

The procedure was as described by the manufacturer (Randox Laboratories, UK). 500 µl of serum, control were dispensed into appropriately labeled specimens and controls tubes respectively. 1000 µl of HDL precipitant (phosphotungstic acid- 0.55 mmol/L and magnesium ions- 25 mmol/L) was added to each of the tube. This was incubated for 10 minutes at room temperature and then centrifuged at 4000 rpm for 10 minutes. 100 µl of the clear supernatant was used to determine cholesterol content by cholesterol assay mothod. The HDL content was then calculated.

3.10.19 Quantitative determination of serum Low Density Lipoprotein Cholesterol (LDL-C) as described by Kaplan *et al* (1983).

The formula by Kaplan and colleagues (1983) was used to calculate the LDL-C level. Initially, the total cholesterol, triglyceride and HDL-C levels of each sample were determined and the LDL level was calculated using this formula: LDL-C = Total cholesterol – (HDL-C + 1/5 x triglyceride). The formula hinges on the postulation that VLDL-C is present in a concentration equal to one fifth of the triglyceride concentration.

3.11 Statistical analysis

The results from this study were statistically analysed using Students't-test, one way analysis of variance (ANOVA) and Pearson r correlation were used to compare means. The analyses were performed with the use of Statistical *Package for Social Sciences* (SPSS) statistical software package, version 16.0. $P < 0.05$ is considered statistically significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Apolipoprotein and Lipid profiles in symptomatic HIV infected subjects on ART, Symptomatic HIV without ART, Asymptomatic HIV subjects and Control group.

4.1.1 Apolipoprotein profile:

The serum Apolipoprotein A_1, Apo A_2, Apo B, Apo C_2, Apo C_3 and Apo E (g/l) levels showed significantes different amongst the groups at $P <0.05$ (F = 474.03; 52.13; 128.50; 9.80; 27.70 and 131.12) respectively. Pairwise comparisons showed that the serum Apo A_1, Apo B, Apo $C_2$and Apo C_3 levels were significantly higher in symptomatic HIV infected subjects on ART compared to symptomatic HIV infected subjects who were not on ART ($p<0.05$, in each case). However, the serum Apo A_2 and Apo E levels were significantly lower in symptomatic HIV infected subjects on ART compared to symptomatic HIV infected subjects not on ART ($p<0.05$, in each case). Similarly, the serum Apo A_1 and Apo C_2 levels were significantly higher in symptomatic HIV infected subjects on ART compared to asymptomatic HIV infected subjects ($p<0.05$, in each case). But, the serum Apo A_2 was significantly lower in symptomatic HIV infected subjects on ART compared to symptomatic HIV infected subjects not on ART ($p<0.05$). However, the serum Apo B, Apo C_3 and Apo E levels were not significantly different in symptomatic HIV infected subjects on ART compared to asymptomatic HIV infected subjects ($p>0.05$, respectively).

Pairwise comparisons showed that the serum Apo A_1, Apo A_2, Apo B, Apo C_2 and Apo E levels were significantly higher in symptomatic HIV infected subjects on ART compared to HIV seronagative control subjects at $p<0.05$ respectively. But, no significant difference was observed in the serum level of Apo C_3 in symptomatic HIV infected subjects on ART compared to HIV seronagative control subjects at $p>0.05$.

Again, pairwise comparisons showed that the serum Apo A_2, Apo B, Apo C_2 and Apo E levels were significantly higher in symptomatic HIV infected subjects not on ART compared to asymptomatic HIV infected subjects at $p<0.05$ respectively. However, the serum Apo A_1

and Apo C_3 were significantly lower in symptomatic HIV infected subjects not on ART compared to asymptomatic HIV infected subjects ($p<0.05$, in each case).

Similarly, the serum Apo A_2, Apo B and Apo E levels were significantly higher in symptomatic HIV infected subjects not on ART compared to HIV seronagative control subjects at $p<0.05$ respectively. But, the serum Apo A_1 and Apo C_3 levels were significantly lower in symptomatic HIV infected subjects not on ART compared to HIV seronegative control subjects at $p<0.05$ respectively. However, the serum Apo C_2 was the same in symptomatic HIV infected subjects not on ART compared to HIV seronegative control subjects at $P>0.05$.

Again, the serum Apo A_2, Apo B and Apo E levels were significantly higher in asymptomatic HIV infected subjects compared to HIV seronegative control subjects ($p<0.05$, in each case). But, the serum Apo A_1 and Apo C_2 levels were significantly lower in asymptomatic HIV infected subjects compared to HIV seronegative control subjects ($p<0.05$, in each case). However, the serum Apo C_3 was the same in asymptomatic HIV infected subjects compared to HIV seronegative control subjects ($P>0.05$).

4.1.2 Lipid profile:

The serum total Cholesterol, LDL, HDL and Triglyceride (mmol/l) levels showed significant differenes amongst the groups at $P< 0.05$ ($F = 107.10$; 400.03; 272.4 and 911.00) respectively. Pairwise comparisons showed that the serum total Cholesterol, LDL and HDL levels were significantly higher in symptomatic HIV infected subjects on ART compared to symptomatic HIV infected subjects not on ART at $p<0.05$ respectively. However, a lower serum level of Triglyceride was seen in symptomatic HIV infected subjects not on ART compared to symptomatic HIV infected subjects on ART ($p<0.05$).

Also, pairwise comparison showed that the serum total Cholesterol, LDL and Triglyceride levels were significantly higher in symptomatic HIV infected subjects on ART compared to asymptomatic HIV infected subjects at $p<0.05$, in each case. But a lower serum level of HDL was observed in symptomatic HIV infected subjects not on ART compared to symptomatic

HIV infected subjects on ART ($p<0.05$). Similarly, the serum total Cholesterol, LDL and Triglyceride levels were significantly higher in symptomatic HIV infected subjects on ART compared to asymptomatic HIV control subjects at $p<0.05$ respectively. But, a lower serum level of HDL was seen in symptomatic HIV infected subjects on ART compared to asymptomatic HIV control subjects at $p<0.05$.

Pairwise comparisons showed that the serum total Cholesterol, LDL, HDL and Triglyceride levels were significantly lower in symptomatic HIV infected subjects not on ART compared to asymptomatic HIV infected subjects ($p<0.05$, in each case). Again, pairwise comparisons showed that the serum total Cholesterol and HDL levels were significantly lower in symptomatic HIV infected subjects not on ART compared to HIV seronegative control subjects at $p<0.05$ respectively. But a significantly higher serum levels of LDL and Triglyceride were seen in symptomatic HIV infected subjects not on ART compared to HIV seronegative control subjects ($p<0.05$, in each case). Pairwise comparisons showed that the serum total Cholesterol, HDL and Triglyceride levels were significantly lower in asymptomatic HIV infected subjects compared to HIV seronegative control subjects at $p<0.05$ respectively. But a significantly higher serum level of LDL was seen in asymptomatic HIV infected subjects compared to HIV seronegative control subjects at $p<0.05$ (See table 4.1).

Table 4.1: Comparison of mean ± SD serum levels of Apolipoprotein and Lipid profiles in symptomatic HIV infected subjects on ART (A), symptomatic HIV without ART (B), Asymptomatic HIV subjects (C) and control group (D).

Group(n=100)	Apo A-1(g/L)	Apo A-1 1(g/L)	Apo B(g/L)	Apo C-11(g/L)	Apo C-111 (g/L)	Apo E (g/L)	T Chol (mmol/L)	LDL (mmol/L)	HDL (mmol/L)	TG (mmol/L)	TG (mmol/L)
Symptomatic HIV on ART (A)	1.08 ± 0.24	0.30 ± 0.11	1.47 ± 0.69	0.06 ±0.05	0.03 ± 0.02	0.10 ± 0.06	5.72±0.43	3.71±0.21	1.12±0.21	1.75±0.04	1.75±0.04
Symptomatic HIV without ART (B)	0.35 ± 0.20	0.55 ± 0.34	2.84 ± 0.89	0.05 ± 0.02	0.01 ± 0.01	0.24 ± 0.11	3.51±0.22	1.75±0.06	0.91±0.06	0.79±0.04	0.79±0.04
Asymptomatic HIV(C)	0.60 ± 0.23	0.44 ± 0.13	1.50 ± 0.02	0.04 ± 0.01	0.03 ± 0.01	0.11 ± 0.07	4.27±0.15	2.76±0.07	1.20±0.03	1.35 ±0.04	1.35±0.04
Control (D)	1.26 ± 0.06	0.24 ± 0.09	0.68 ± 0.29	0.05 ± 0.02	0.03 ± 0.02	0.05 ± 0.01	4.62±0.24	2.34±0.13	1.37±0.06	1.44 ± 0.05	1.44±0.05
F-value	474.03	52.13	128.50	9.80	27.70	131.12	107.10	400.03	272.40	912.00	
p-value	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	912.00 (.000)
A v B	<0.05	<0.05	>0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
A v C	<0.05	<0.05	<0.05	<0.05	>0.05	>0.05	<0.05	<0.05	<0.05	<0.05	<0.05
A v D	<0.05	<0.05	<0.05	<0.05	>0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
B v C	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
B v D	<0.05	<0.05	<0.05	>0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
C v D	<0.05	<0.05	<0.05	<0.05	>0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

4.2 Apolipoprotein and Lipid profiles in symptomatic HIV infected subjects on ART, in symptomatic HIV infected subjects without ART, in Asymptomatic HIV infected subjects and Control subjects with malaria parasitaemia and without malaria parasitaemia in all groups.

4.2.1 Apolipoprotein and Lipid profiles in Symptomatic HIV infected subjects on ART with malaria parasitaemia and without malaria parasitaemia:

Pairewise comparisons showed that the serum Apo C_3 was significantly higher in symptomatic HIV infected subjects on ART with malaria parasitaemia compared to symptomatic HIV infected subjects on ART without malaria parasitaemia at $p<0.05$. But, the mean serum Apo E and HDL levels were significantly lower in symptomatic HIV infected subjects on ART with malaria parasitaemia compared to symptomatic HIV infected subjects on ART without malaria parasitaemia at $p<0.05$. However, the serum Apo A_1, Apo A_2, Apo B, Apo C_2, T Chol, LDL and TG values were the same in symptomatic HIV infected subjects on ART with malaria parasitaemia compared to symptomatic HIV infected subjects on ART without malaria parasitaemia ($p>0.05$ respectively).

4.2.2 Apolipoprotein and Lipid profiles in Symptomatic HIV infected subjects not on ART with malaria parasitaemia and without malaria parasitaemia:

Pairewise comparisons showed that the serum levels of Apo E and TG were significantly higher in symptomatic HIV infected subjects without ART with malaria infection compared to symptomatic HIV infected subjects without ART without malaria infection at $p<0.05$ respectively. But, the serum levels of Apo C_2 and HDL were significantly lower in symptomatic HIV infected subjects without ART with malaria infection compared to symptomatic HIV infected subjects without ART without malaria infection at $p<0.05$ respectively. However, the values of serum Apo_1, Apo A_2, Apo B, Apo C_3, T Chol and LDL were the same in symptomatic HIV infected subjects without ART with malaria infection compared to symptomatic HIV infected subjects without ART without malaria infection at $p>0.05$ respectively.

4.2.3 Apolipoprotein and Lipid profiles in Aymptomatic HIV infected subjects with malaria parasitaemia and without malaria parasitaemia:

Pairewise comparisons showed that the serum Apo E was significantly higher in asymptomatic HIV infected subjects with malaria parasitaemia compared to asymptomatic HIV infected subjects without malaria parasitaemiaat $p<0.05$. However, the serum Apo A_1, Apo A_2, Apo B, Apo C_2, Apo C_3, T Chol, HDL, LDL and TG values were the same in asymptomatic HIV infected subjects with malaria parasitaemia compared to asymptomatic HIV infected subjects without malaria parasitaemia ($p>0.05$ respectively).

4.2.4 Apolipoprotein and Lipid profiles in Control subjects with malaria parasitaemia and without malaria parasitaemia:

Pairewise comparisons showed that the serum level of LDL was significantly lower in control subjects with malaria infection compared to control subjects without malaria infection at $p<0.05$. However, the values of serum Apo_1, Apo A_2, Apo B, Apo C_2, Apo C_3, T Chol, HDL and TG were the same in control subjects with malaria infection compared to control subjects without malaria infection at $p>0.05$ respectively (see table 4.2).

4.3 cardiac markers in Symptomatic HIV infected subjects on ART, Symptomatic HIV without ART, Asymptomatic HIV subjects and Control group.

4.3 Myoglobin and Troponin I levels:

The serum Myoglobin and Troponin I (ng/mL) levels were significantly different amongst the groups at $P < 0.05$ ($F = 20.52$ and 112.50) respectively. Pairewise comparisons showed that the serum Myoglobin and Troponin I levels were significantly lower in symptomatic HIV infected subjects on ART compared to symptomatic HIV infected subjects not on ART at $p<0.05$ respectively. Again, between group comparison showed that the serum Troponin I was significantly higher in symptomatic HIV infected subjects on ART compared to asymptomatic HIV infected subjects at $p<0.05$. However, there was no significant difference

in serum Myoglobin observed in symptomatic HIV infected subjects on ART compared to asymptomatic HIV infected subjects at $p>0.05$.

Similarly, the serum Troponin I level was significantly higher in symptomatic HIV infected subjects on ART compared with to HIV seronagetive control subjects ($P<0.05$). Also, the serum Myoglobin and Troponin I levels were significantly higher in symptomatic HIV infected subjects not on ART compared to asymptomatic HIV infected subjects ($P<0.05$, in each case). Again, the serum Myoglobin and Troponin I levels were significantly higher in symptomatic HIV infected subjects not on ART compared to HIV seronegative control subjects ($P<0.05$, in each case). Similarly, the serum Troponin I level was significantly higher in asymptomatic HIV infected subjects compared to HIV seronegative control subjects ($P<0.05$). However, the serum levels of Myoglobin were the same in symptomatic HIV infected subjects on ART and in asymptomatic HIV infected subjects compared to HIV seronegative control subjects ($P<0.05$, in each case).

4.3.2 Cardiac markers and CD4 counts:

The serum activities of total CK, CK-MB, LDH,AST (IU/L) levels and CD4 counts were significant different amongst the groups at $P < 0.05$ ($F = 181.17$; 343.38, 402.60, 115.44 and 123.10) respectively. Between group comparison showed that the serum activities of total CK, CK-MB and AST levels were significantly lower in symptomatic HIV infected subjects on ART compared to symptomatic HIV infected subjects not on ART at $p<0.05$ respectively. But the serum activity of LDL was not significantly different in symptomatic HIV infected subjects on ART compared to symptomatic HIV infected subjects not on ART at $p>0.05$.

Again, pairewise comparisons showed that the serum activities of CK-MB and LDH levels were significantly higher in symptomatic HIV infected subjects on ART compared to asymptomatic HIV infected subjects ($P<0.05$, in each case). However, the serum activities of total CK and AST levels were significantly lower in symptomatic HIV infected subjects on ART compared to asymptomatic HIV infected subjects ($P<0.05$, in each case). Between group comparison showed that the serum activities of total CK, CK-MB, LDH and AST

levels were significantly higher in symptomatic HIV infected subjects on ART compared to HIV seronegative control subjects at $P<0.05$ respectively; in symptomatic HIV infected subjects not on ART compared to asymptomatic HIV infected subjects ($P<0.05$, in each case) and in symptomatic HIV infected subjects not on ART compared to HIV seronegative control subjects ($P<0.05$,respectively). Also, between group comparison showed that the serum activities of total CK, CK-MB and AST levels were significantly higher in asymptomatic HIV infected subjects compared to HIV seronegative control subjects($P<0.05$,respectively). But, no significant difference in serum activities of LDH was observed in asymptomatic HIV infected subjects compared to HIV seronegative control subjects ($P<0.05$).

Pairewise comparisons showed that the mean $CD4^+$ T-Cell count was significantly higher in symptomatic HIV infected subjects on ART compared to symptomatic HIV infected subjects not on at $p<0.05$. Again, the $CD4^+$ T-Cell count was significantly higher in symptomatic HIV infected subjects on ART compared to asymptomatic HIV infected subjects ($p<0.05$).The $CD4^+$ T-Cell count was significantly lower in symptomatic HIV infected subjects on ART compared to HIV seronegative control subjects; in symptomatic HIV infected subjects not on ART compared to asymptomatic HIV infected subjects; in symptomatic HIV infected subjects not on ART compared to HIV seronegative control subjects and in asymptomatic HIV infected subjects compared to HIV seronegative control subjects ($p<0.05$ respectively) (See table 4.3)

Table 4.2: Mean ± SD serum levels of Apolipoprotein and Lipid profiles in symptomatic HIV infected subjects on ART, without ART, in Asymptomatic HIV infected subjects and Control subjects each with and without malaria parasitaemia

Groups	Apo A-1 (g/L)	Apo A-11 (g/L)	Apo B (g/L)	Apo C-11 (g/L)	Apo C-111 (g/L)	Apo E (g/L)	T Chol (mmol/L)	LDL (mmol/L)	HDL (mmol/L)	TG (mmol/L)
Symptomatic HIV on ART mp^+ (n=50)	1.08 ± 0.23	0.28 ± 0.01	1.44 ± 0.72	0.07 ± 0.05	0.04 ± 0.02	0.09 ± 0.06	5.57 ± 0.46	3.67 ± 0.27	0.95 ± 0.15	1.08 ± 0.23
Symptomatic HIV on ART mp- (n=50)	1.09 ± 0.25	0.30 ± 0.12	1.50± 0.65	0.06 ± 0.05	0.02 ± 0.01	0.12 ± 0.07	5.86 ± 0.34	3.75 ± 0.10	1.30 ± 0.05	1.09 ± 0.25
p-value	>0.05	>0.05	>0.05	>0.05	<0.05	<0.05	>0.05	>0.05	<0.05	>0.05
Symptomatic HIV without ART mp^+ (n=50)	0.30 ± 0.19	0.85 ± 0.20	2.50 ± 1.00	0.04 ± 0.02	0.01 ± 0.01	0.27 ± 0.12	3.51± 0.17	1.73 ± 0.02	0.86 ± 0.04	1.77 ± 0.03
Symptomatic HIV without ART mp- (n=50)	0.40 ± 0.20	0.83 ± 0.21	2.32± 0.72	0.06 ± 0.02	0.01± 0.01	0.21 ± 0.09	3.52 ± 0.26	1.78 ± 0.07	0.97 ± 0.03	0.81 ± 0.04
p-value	>0.05	>0.05	>0.05	<0.05	>0.05	<0.05	>0.05	>0.05	<0.05	<0.05
Asymptomatic HIV mp^+ (n=50)	0.61 ± 0.24	0.45 ± 0.14	1.44 ± 0.52	0.04 ± 0.01	0.02 ± 0.02	0.14 ± 0.08	4.18± 0.11	2.75 ± 0.07	1.19 ± 0.03	1.32± 0.02
Asymptomatic HIV mp- (n=50)	0.58± 0.14	0.43 ± 0.12	1.55± 0.38	0.05 ± 0.01	0.03± 0.02	0.08 ± 0.03	4.32 ± 0.12	2.77 ± 0.06	1.20 ± 0.04	1.37 ± 0.03
p-value	>0.05	>0.05	>0.05	>0.05	>0.05	<0.05	>0.05	>0.05	>0.05	>0.05
Control subjects mp^+ (n=50)	1.27 ± 0.06	0.25 ± 0.11	0.62 ± 0.26	0.05 ± 0.02	0.03 ± 0.02	0.05 ± 0.01	4.64± 0.26	2.44 ± 0.77	1.32 ± 0.05	1.41± 0.02
Control subjects mp- (n=50)	1.28± 0.53	0.23 ± 0.04	0.73± 0.31	0.05 ± 0.02	0.03± 0.02	0.04 ± 0.01	5.67 ± 5.41	2.77 ± 0.05	1.41± 0.05	1.47 ± 0.05
p-value	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	<0.05	>0.05	>0.05

Table 4.3: Mean ± SD serum levels of Cardiac markers and CD4 in symptomatic HIV infected subjects on ART, without ART, Asymptomatic HIV subjects and control group.

Group(n=100)	Myoglobin (ng/mL)	Troponin (ng/mL)	CK-T (IU/L)	CK-MB (IU/L) (IU/L)	LDH (IU/L)	AST	CD4 (/µL)
Symptomatic HIV on ART (A)	40.75 ± 39.98	1.38 ± 0.15	95.85 ± 7.76	7.57 ± 2.12 23.11 ± 5.46	185.58 ± 23.92		619.58 ± 392.74
Symptomatic HIV without ART (B)	60.40 ± 32.87	1.60 ± 0.25	122.50 ± 17.59	13.96 ± 4.35 31.63 ± 9.28	187.22 ± 25.25		374.78 ± 121.59
Asymptomatic HIV(C)	41.43 ± 13.74	0.66 ± 0.16	102.28 ± 16.79	4.88 ± 1.64 20.23 ± 8.64	157.12 ± 25.25		437.20 ± 129.75
Control (D)	30.12 ± 15.03	0.03 ± 0.16	60.84 ± 28.32	2.51 ± 1.51 12.27 ± 5.57	155.20 ± 23.25		940.64 ± 148.85
				343.38 115.44	402.60		
F-value	20.52	112.50	128.50	<0.05	<0.05		123.10
p-value	<0.05	<0.05	<0.05	<0.05			<0.05
				<0.05	>0.05		
A v B	<0.05	<0.05	>0.05	<0.05			<0.05
				<0.05	<0.05		
A v C	>0.05	<0.05	<0.05	<0.05			>0.05
				<0.05	<0.05		
A v D	>0.05	<0.05	<0.05	<0.05			<0.05
				<0.05	<0.05		
B v C	<0.05	<0.05	<0.05	<0.05			<0.05
				<0.05	<0.05		
B v D	<0.05	<0.05	<0.05	<0.05			<0.05
				<0.05	>0.05		
C v D	>0.05	<0.05	<0.05	<0.05			<0.05

4.4 Cardiac markers and CD4 in symptomatic HIV infected subjects on ART, without ART, in Asymptomatic HIV infected subjects and Control subjects with and without malaria parasitaemia

4.4.1 Cardiac markers and CD4 in Symptomatic HIV infected subjects on ART with malaria parasitaemia and without malaria parasitaemia:

Pairewise comparisons showed that serum activity of CK-MB was significantly higher in symptomatic HIV infected subjects on ART with malaria parasitaemia compared to symptomatic HIV infected subjects on ART without malaria parasitaemia at $p<0.05$. But, the CD4 count was significantly lower in symptomatic HIV infected subjects on ART with malaria infection compared to symptomatic HIV infected subjects on ART without malaria infection at $p<0.05$. However, the values of Myoglobin, Troponin; the serum activities of CK-T, LDH and AST were the same in symptomatic HIV infected subjects on ART with malaria infection compared to symptomatic HIV infected subjects on ART without malaria infection at $p>0.05$ respectively.

4.4.2 Cardiac markers and CD4 in Symptomatic HIV infected subjects without ART with and without malaria parasitaemia:

Pairewise comparisons showed that the serum levels of CK-T, CK-MB, LDH, AST and blood CD4 count were the same in symptomatic HIV infected subjects without ART with malaria infection compared to symptomatic HIV infected subjects without ART without malaria infection at $p>0.05$ respectively. But the serum level of myoglobin was significantly higher in symptomatic HIV infected subjects without ART with malaria infection compared to those without malaria infection at $p>0.05$.

4.4.3 Cardiac markers and CD4 in Asymptomatic HIV infected subjects with and without malaria parasitaemia:

Pairewise comparisons showed that the serum level of troponin I was significantly higher in asymptomatic HIV infected subjects with malaria parasitaemia compared to asymptomatic HIV infected subjects without malaria parasitaemiaat $p<0.05$. However, the serum levels of

CK-T, CK-MB, LDH, AST, myoglobin and blood CD4 count were the same in asymptomatic HIV infected subjects with malaria parasitaemia compared to asymptomatic HIV infected subjects without malaria parasitaemia ($p>0.05$ respectively).

4.4.4 Cardiac markers and CD4 in Control subjects with and without malaria parasitaemia:

Pairewise comparisons showed that the serum levels of Myoglobin, Troponin I, CK-T, CK-MB and LDH were significantly higher in control subjects with malaria infection compared to control subjects without malaria infection at $p<0.05$ respectively. But, the values of AST and CD4 were the same in control subjects with malaria infection compared to control subjects without malaria infection at $p>0.05$ respectively. Also, pairewise comparisons showed that the serum levels of LDH was significantly lower in control subjects with malaria infection compared to control subjects without malaria infection ($p<0.05$). Also, significant differences were observed in levels of Myoglobin, Troponin, CK-T and CK-MB in control subjects with malaria infection compared to control subjects without malaria infection ($p<0.05$, in each case) (see table 4.4).

Table 4.4: Mean ± SD serum levels of Cardiac markers and CD4 in symptomatic HIV infected subjects on ART,without ART, in Asymptomatic HIV infected subjects and Control subjects with and without malaria parasitaemia

Groups	Myoglobin (ng/mL)	Troponin (ng/mL)	CK-T (IU/L)	CK-MB (IUL)	LDH (IU/L)	AST (IU/L)	CD4 (/µL)
Symptomatic HIV on ART mp+ (n=50)	43.60 ± 41.30	1.46 ± 0.15	97.10 ± 7.52	8.37 ± 1.83	189.46 ± 24.00	23.60 ± 5.66	535.58 ± 368.91
Symptomatic HIV on ART mp- (n=50)	37.90 ± 38.82	1.31 ± 0.11	94.60± 7.87	6.78 ± 2.11	185.70 ± 23.93	22.61 ± 5.27	706.58 ± 400.11
p-value	>0.05	>0.05	>0.05	<0.05	>0.05	>0.05	<0.05
Symptomatic HIV without ART mp+ (n=50)	76.72 ± 36.82	1.72 ± 0.22	128.9 ± 18.40	16.03 ± 4.04	197.50 ± 21.63	35.28 ± 5.81	390.06 ± 130.75
Symptomatic HIV without ART mp- (n=50)	44.08 ± 16.85	1.47 ± 0.22	116.02± 14.20	11.88 ± 3.64	176.94 ± 24.58	27.99 ± 10.64	359.50 ± 110.90
p-value	<0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
Asymptomatic HIV mp+ (n=50)	39.87 ± 13.14	0.77 ± 0.14	106.50 ± 14.54	5.48 ± 1.57	157.78 ± 25.49	22.97 ± 8.78	416.18 ± 130.47
Asymptomatic HIV mp- (n=50)	42.99 ± 14.27	0.54 ± 0.04	98.06 ± 17.85	4.28 ± 1.50	156.46 ± 25.25	17.49 ± 7.65	457.92 ± 126.94
p-value	>0.05	<0.05	>0.05	>0.05	>0.05	>0.05	>0.05
Control subjects mp+ (n=50)	37.48 ± 14.75	0.35 ± 0.15	68.18 ± 24.54	3.10 ± 1.51	163.36 ± 0.93	14.03 ± 5.34	935.02 ± 151.15
Control subjects mp- (n=50)	22.77 ± 11.33	0.26 ± 0.15	53.50 ± 30.13	1.92 ± 1.27	147.04 ± 23.11	10.52 ± 4.99	946.26 ± 147.83
p-value	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	>0.05

Apolipoprotein profile in HIV infected subjects before Antiretroviral therapy and after commencement of therapy followed up for 12 months.

Apolipoprotein Apo A_1:

The serum Apo A_1 (g/l) level in symptomatic HIV infected subjects before Antiretroviral therapy and after commencement of therapy followed up for 3, 6, 9 and 12 months were 0.04±0.17, 1.16±0.16, 1.34±0.10 and 1.47±0.09 respectively. Pairewise comparisons showed that the serum level Apo A_1 was significantly lower in symptomatic HIV infected subjects before therapy compared to when on 3, 6, 9 and 12 months therapy ($p<0.05$,in each case). Also, the Apo A_1 level was significantly lower in symptomatic HIV infected subjects on 3 months therapy compared to HIV infected subjects on 6, 9 and 12 months therapy ($p<0.05$, in each case). Again, the serum Apo A_1 level was significantly lower in symptomatic HIV infected subjects on 6 months therapy compared to 9 and 12 months therapy ($p<0.05$,in each case). Similarly, the serum Apo A_1 level was significantly lower in symptomatic HIV infected subjects on 9 months therapy compared to symptomatic HIV infected subjects on 12 months therapy ($p<0.05$).

Apolipoprotein Apo A_2:

The serum Apo A_2 (g/L) levels in HIV infected subjects before therapy and when on 3, 6, 9 and 12 months therapy were: 0.85±0.19, 0.13±0.05, 0.27±0.19, 0.28±0.03 and 0.39±0.31 respectively. Pairewise comparisons showed that the Apo A_2 level was significantly higher in symptomatic HIV infected subjects before therapy compared to when they were on 3, 6, 9 and 12 months therapy ($p<0.05$, in each case). However, the serum level of Apo A_2 was significantly lower in symptomatic HIV infected subjects on 3 months therapy compared to when they were on 6, 9 and 12 months therapy at $p>0.05$ respectively. But the Apo A_2 level in symptomatic HIV infected subjects on 6 months was the same compared to symptomatic HIV infected subjects on 9 and 12 months at $p>0.05$ respectively. Also the mean Apo A_2 level in symptomatic HIV infected subjects on 9 months was the same compared to when on 12 months at $p>0.05$ respectively (see figure 4.1).

Apolipoprotein Apo B:

The Apo B (g/L) levels in symptomatic HIV infected subjects before therapy and when on 3, 6, 9, 12 months therapy were: 2.31±0.72, 3.31±0.41, 2.93±0.41, 2.62±0.53 and 1.59 ± 0.49 respectively. Pairewise comparisons showed that the mean Apo B level in symptomatic HIV infected subjects before therapy was significantly lower compared to when on 3, 6 and 9 months therapy ($p<0.05$, in each case). But the Apo B level in symptomatic HIV infected subjects before therapy was significantly higher compared to when on 12 months therapy ($p<0.05$) . The Apo B level in symptomatic HIV infected subjects on 3 month therapy was significantly higher compared to when on 6, 9 and 12 months therapy ($p <0.05$ respectively). Similarly the serum Apo B level in symptomatic HIV infected subjects on 6 months therapy was significantly higher compared to when on 9 and 12 months therapy at $p <0.05$ respectively. Again, the serum Apo B level in symptomatic HIV infected subjects on 9 months therapy was significantly higher compared to when on 12 months therapy ($p <0.05$ (see figure 4.2).

Apolipoprotein Apo C_2:

The serum Apo C_2 (g/l) levels in symptomatic HIV infected subjects before and when on 3, 6, 9 and 12 months therapy were: 0.05±0.02, 0.00±0.00, 0.01±0.00, 0.02±0.00 and 0.02±0.01 respectively. Between group comparison showed that the serum Apo C_2 level was significantly higher in symptomatic HIV infected subjects before therapy compared to when on 3, 6, 9 and 12 months therapy ($p <0.05$ respectively). However, the Apo C_2 level in symptomatic HIV infected subjects before therapy was the same compared to when on 12 months therapy at $p>0.05$. The serum Apo C_2 level was significantly lower in symptomatic HIV infected subjects on 3 months therapy compared to when on 6, 9 and 12 months therapy. Again, the serum Apo C_2 level in symptomatic HIV infected subjects on 6 months therapy was significantly lower compared to when on 9 and 12 months therapy ($p <0.05$ respectively). Nevertheless, the serum Apo C_2 level in symptomatic HIV infected subjects on

9 months therapy was significantly different compared to when on 12 months therapy ($p < 0.05$) (see figure 4.3).

Apolipoprotein Apo C_3:

The serum Apo C_3 (g/l) levels in symptomatic HIV infected subjects before therapy and after 3, 6, 9 and 12 months therapy were: 0.01±0.01, 0.08±0.01, 0.06±0.01, 0.05±0.01 and 0.04±0.01 respectively. Pairewise comparisons showed that the serum Apo C_3 level was significantly lower in symptomatic HIV infected subjects before therapy compared to when on 3, 6, 9 and 12 months therapy at $P<0.05$ respectively. But the serum Apo C_3 level was significantly higher in symptomatic HIV infected subjects on 3 months compared to when on 6, 9 and 12 months therapy at $P<0.05$ respectively. Similarly, the serum Apo C_3 level was significantly higher in symptomatic HIV infected subjects on 6 months compared to when on 9 and 12 months at $P<0.05$ respectively. Again , the serum serum Apo C_3 in symptomatic HIV infected subjects on 9 months therapy was significantly higher compared to when on 12 months therapy at $P<0.05$(see figure 4.4).

Apolipoprotein Apo E:

The serum Apo E (g/L) level in symptomatic HIV infected subjects before therapy and at 3, 6, 9 and 12 months therapy were 0.22±0.08, 0.01±0.01, 0.01±0.01, 0.02±0.01 and 0.03±0.01 respectively. Pairewise comparisons showed that the serum Apo E level was significantly higher in symptomatic HIV infected subjects before therapy compared to when on 3, 6, 9 and 12 months therapy at $P<0.05$ respectively. The serum Apo E level in symptomatic HIV infected subjects on 3 months therapy was significantly the same when compared to when on 6 months therapy. But The serum Apo E level in symptomatic HIV infected subjects on 3 months therapy was significantly lower when compared to when on 9 and 12 months therapy at $P<0.05$ respectively . But the serum Apo E level in symptomatic HIV infected subjects on 6 months therapy was significantly lower compared to when on 9 and 12 months therapy at $P<0.05$ respectively. Also, the serum Apo E level in symptomatic HIV infected subjects on 9

months therapy was significantly lower compared to when on 12 months therapy at $P<0.05$ (see figure 4.1) (see figure 4.5).

Apolipoprotein profile studied between sex distributions in symptomatic HIV infected subjects before Antiretroviral therapy and after commencement of therapy followed up for 12 months.

Apolipoprotein Apo A_1:

Pairewise comparisons showed that the serum Apo A_1 level in male symptomatic HIV infected subjects before antiretroviral therapy was significantly lower compared to female symptomatic HIV infected subjects before antiretroviral therapy ($p<0.05$). Again, serum Apo A_1 level in male symptomatic HIV infected subjects on 3 months antiretroviral therapy was significantly lower compared to female symptomatic HIV infected subjects on 3 months antiretroviral therapy ($p<0.05$) (see figure 4.6).

Apolipoprotein Apo A_2:

The serum Apo A_2 level was the same between sex compared with symptomatic HIV infected subjects before antiretroviral therapy and after commencement of therapy followed up for 12 months ($p>0.05$) (see figure 4.7).

Apolipoprotein Apo B:

The serum Apo B level was the same between sex compared with symptomatic HIV infected subjects before antiretroviral therapy and after commencement of therapy followed up for 12 months ($p>0.05$) (see figure 4.8).

Apolipoprotein Apo C_2:

The serum Apo C_2 level was the same between sex compared with symptomatic HIV infected subjects before antiretroviral therapy and after commencement of therapy followed up for 12 months ($p>0.05$) (see figure 4.9).

Apolipoprotein Apo C_3:

The serum Apo C_3 level was the same between sex compared with symptomatic HIV infected subjects before antiretroviral therapy and after commencement of therapy followed up for 12 months (p>0.05) (see figure 4.10).

Apolipoprotein Apo E:

The serum Apo E level was the same between sex compared with symptomatic HIV infected subjects before antiretroviral therapy and after commencement of therapy followed up for 12 months (p>0.05) (see figure 4. 11).

Lipid profile in HIV infected subjects before Antiretroviral therapy and after commencement of therapy followed up for 12 months.

Total cholesterol:

The serum total cholesterol level in symptomatic HIV infected subjects before therapy and when on 3, 6, 9 and 12 months therapy were: 3.43±0.28, 3.92±0.46, 4.55±0.88, 4.82±0.93 and 5.57±1.31 respectively. Pairewise comparisons showed that the level of Total cholesterol was significantly lower in symptomatic HIV infected subjects before therapy compared to when on 3, 6, 9 and 12 months therapy at P<0.05 respectively. Similarly, the serum Total cholesterol level in symptomatic HIV infected subjects on 3 months was significantly lower compared to when on 6, 9 and 12 months therapy at P<0.05 respectively . Again, the serum Total cholesterol level was significantly lower in symptomatic HIV infected subjects on 6 months compared to when on 9 and 12 months at P<0.05 respectively. Also, the serum Total cholesterol in symptomatic HIV infected subjects on 9 months therapy was significantly lower compared to when on 12 months ART at P<0.05.

Low density lipoprotein:

The serum LDL (mmol/L) levels in symptomatic HIV infected subjects before therapy and when on 3, 6, 9 and 12 months therapy were: 2.37±0.24, 2.85±0.46, 3.13±0.98, 3.17±1.06 and 3.68±1.34 respectively. Pairewise comparisons showed that the serum LDL level was significantly lower in HIV infected subjects before therapy compared to when on 3, 6, 9 and

12 months therapy at $P<0.05$ respectively. Similarly, the serum LDL level in symptomatic HIV infected subjects on 3 months therapy was significantly lower compared to when on 6, 9 and 12 months therapy at $p<0.05$ respectively. Also the serum LDL level in symptomatic HIV infected subjects on 6 month therapy was significantly lower compared to when on 9 and 12 months therapy at $p<0.05$ respectively. Again, the serum LDL level in symptomatic HIV infected subjects on 9 months therapy was significantly lower compared to when on 12 months therapy at $p<0.05$.

High density lipoprotein:

The serum HDL (mmol/L) level in symptomatic HIV infected subjects before therapy and when on 3, 6, 9 and 12 months therapy were: 0.96±0.04, 1.08±0.96, 1.29±0.70, 1.36±0.71 and 1.64±0.61 respectively. Pairewise comparisons showed that the serum HDL level was significantly lower in symptomatic HIV infected subjects before therapy compared to when on 3, 6, 9 and 12 months therapy at $P<0.05$ respectively. Similarly, the serum HDL level in symptomatic HIV infected subjects on 3 months therapy was significantly lower compared to when on 6, 9 and 12 months therapy at $p<0.05$ respectively. Also the serum HDL level in symptomatic HIV infected subjects on 6 month therapy was significantly lower compared to when on 9 and 12 months therapy at $p<0.05$ respectively. Again, the serum HDL level in symptomatic HIV infected subjects on 9 months therapy was significantly lower compared to when on 12 months therapy at $p<0.05$.

Triglyceride:

The serum TG (mmol/L) level in symptomatic HIV infected subjects before therapy and when on 3, 6, 9 and 12 months therapy were: 0.78±0.08, 0.89±0.20, 0.95±0.51, 0.94±0.41, and 0.85±0.43 respectively. Pairewise comparisons showed that the serum TG level in symptomatic HIV infected subjects before therapy was significantly lower compared to when on 3 months therapy at $p<0.05$. Also, the serum TG level in symptomatic HIV infected subjects on 3 months therapy was significantly lower compared to when on 9 months therapy at $p<0.05$. But the serum TG level in symptomatic HIV infected subjects on 3 month therapy

was the same compared to when on 6, 9 and 12 months therapy at p>0.05 respectively. The serum TG level in symptomatic HIV infected subjects on 6 months therapy was the same compared to when on 9 and 12 months therapy at p>0.05 respectively. Similarly, the serum TG level in symptomatic HIV infected subjects on 9 months therapy was the same compared to when on 12 months therapy at p>0.05 (see figure 4.12).

Lipid profile studied between sex distributions in symptomatic HIV infected subjects before Antiretroviral therapy and after commencement of therapy followed up for 12 months.

Total Cholesterol:

The serum total Cholesterol level was the same between sex compared with symptomatic HIV infected subjects before antiretroviral therapy and after commencement of therapy followed up for 12 months (p>0.05) (see figure 4.13).

Low density lipoprotein:

Between sex comparison showed that the serum LDL level in male symptomatic HIV infected subjects before antiretroviral therapy was significantly higher compared to female symptomatic HIV infected subjects before antiretroviral therapy (p<0.05). Again, serum LDL level in male symptomatic HIV infected subjects on 6 months antiretroviral therapy was significantly higher compared to female symptomatic HIV infected subjects on 6 months antiretroviral therapy (p<0.05). Also, serum LDL level in male symptomatic HIV infected subjects on 9 months antiretroviral therapy was significantly higher compared to female symptomatic HIV infected subjects on 9 months antiretroviral therapy (p<0.05). Similarly, serum LDL level in male symptomatic HIV infected subjects on 12 months antiretroviral therapy was significantly higher compared to female symptomatic HIV infected subjects on 12 months antiretroviral therapy (p<0.05) (see figure 4.14).

High density lipoprotein:

Pairewise comparisons showed that the serum HDL level in male symptomatic HIV infected subjects on 3 months antiretroviral therapy was significantly higher compared to female symptomatic HIV infected subjects on 3 months antiretroviral therapy (p<0.05). However,

the serum HDL level in male symptomatic HIV infected subjects on 6 months antiretroviral therapy was significantly lower compared to female symptomatic HIV infected subjects on 6 months antiretroviral therapy. Also, the serum HDL level in male symptomatic HIV infected subjects on 9 months antiretroviral therapy was significantly lower compared to female symptomatic HIV infected subjects on 9 months antiretroviral therapy. Similarly, the serum HDL level in male symptomatic HIV infected subjects on 12 months antiretroviral therapy was significantly lower compared to female symptomatic HIV infected subjects on 12 months antiretroviral therapy (see figure 4.15).

Triglyceride:

Again, pairewise comparisons showed that the serum TG level in male symptomatic HIV infected subjects on 6 months antiretroviral therapy was significantly higher compared to female symptomatic HIV infected subjects on 6 months antiretroviral therapy ($p<0.05$). Also, serum TG level in male symptomatic HIV infected subjects on 9 months antiretroviral therapy was significantly higher compared to female symptomatic HIV infected subjects on 9 months antiretroviral therapy ($p<0.05$). Similarly, serum TG level in male symptomatic HIV infected subjects on 12 months antiretroviral therapy was significantly higher compared to female symptomatic HIV infected subjects on 12 months antiretroviral therapy ($p<0.05$) (see figure 4.16)

Aspartate transferase, Myoglobin, Creatine kinase and Creatine kinase MB in HIV infected subjects before Antiretroviral therapy and after commencement of therapy followed up for 12 months.

Aspartate transferase:

The serum activities AST (U/L) levels in symptomatic HIV infected subjects before therapy and when on 3, 6, 9 and 12 months therapy were 31.82±10.75, 26.18±7.49, 24.74±10.48, 25.70±10.88 and 32.63±21.61 respectively. Pairewise comparisons showed that serum activity AST level was significantly higher in symptomatic HIV infected subjects before therapy compared to when on 3, 6,and 9 months therapy at $p<0.05$ respectively. However,

the serum activity AST level in symptomatic HIV infected subjects before therapy was the same compared to when on 12 months therapy at $p>0.05$. Also, the serum activity AST level in symptomatic HIV infected subjects on 3 months therapy was the same compared to when on 6, 9 and 12 months therapy at p>.0.05 respectively. Similarly, the serum activity AST level in symptomatic HIV infected subjects on 6 month therapy was the same compared to when on 9 and 12 months therapy at p>.0.05 respectively. Again, the serum activity AST level in symptomatic HIV infected subjects on 9 months therapy was the same compared to when on 12 month therapy at p>.0.05 (see figure 4.17).

Myoglobin:

The serum Myoglobin (ng/ml) levels in symptomatic HIV infected subjects before therapy and when on 3, 6, 9 and 12 months therapy were 43.98±15.67, 103.83±68.44, 63.32±21.23, 29.16±11.97 and 17.97±10.92 respectively. Pairewise comparisons showed that the serum Myoglobin level was lower in symptomatic HIV infected subject before therapy compared to when on 3 and 6 months therapy at $p<0.05$ respectively. But the serum Myoglobin level in symptomatic HIV infected subject before therapy was significantly higher compared to when on 9 and 12 months at $p<0.05$ respectively (see figure 4.18).

Total Creatine kinase:

The serum activities of Total CK levels in symptomatic HIV infected subjects before therapy and when on 3, 6, 9 and 12 months therapy were 112.00±14.22, 47.06±25.89, 30.30±9.48, 18.74±5.36 and 12.91±6.91 respectively. Pairewise comparisons showed that serum activity of Total CK level was significantly higher in symptomatic HIV infected subjects before therapy compared to when on 3, 6, 9 and 12 months therapy at $p<0.05$ respectively. Also, the serum activity Total CK level in symptomatic HIV infected subjects at 3 months therapy was significantly higher compared to when on 6, 9 and 12 months therapy at $p<0.05$ respectively. Similarly, the serum activity Total CK level in symptomatic HIV infected subjects on 6 months therapy was significantly higher compared to when on 9 and 12 months therapy at $p<0.05$ respectively. Again, the serum activity Total CK level in symptomatic HIV infected

subjects on 9 months therapy was significantly higher compared to when on 12 months therapy at $p<0.05$ (see figure 4.19).

Creatine kinase MB:

The serum activities CK-MB levels in symptomatic HIV infected subjects before therapy and when on 3, 6, 9 and 12 months therapy were: 10.76±3.52, 29.03±15.07, 19.49±9.12, 10.87±4.13 and 6.93±3.14 respectively. Pairewise comparisons, the serum activity level of CK-MB level was significantly lower in symptomatic HIV infected subjects before therapy compared to when on 3 and 6 months therapy at $p<0.05$ respectively. But the serum activity CK-MB level was the same in symptomatic HIV infected subjects before therapy compared to when on 12 months therapy at $p<0.05$. However, the serum activity CK-MB level was the same in symptomatic HIV infected subjects before therapy compared to when on 9 months therapy at $p<0.05$. The serum activity CK-MB level in symptomatic HIV infected subjects on 6 months therapy was significantly higher compared to when on 9 and 12 months therapy at $p<0.05$ respectively. Also The serum activity CK-MB level in symptomatic HIV infected subjects on 9 months therapy was significantly higher compared to when on 12 months therapy at $p<0.05$ (see fig.4.20).

Aspartate transferase, Myoglobin, Creatine kinase and Creatine kinase MB studied between sex distributions in symptomatic HIV infected subjects before Antiretroviral therapy and after commencement of therapy followed up for 12 months.

Aspartate transferase:

Pairewise comparisons showed that the serum activity of AST in male symptomatic HIV infected subjects before antiretroviral therapy was significantly lower compared to female symptomatic HIV infected subjects before antiretroviral therapy ($p<0.05$ (see figure 4.21).

Myoglobin level:

Pairewise comparisons showed that the serum Myoglobin level in male symptomatic HIV infected subjects on 3 months antiretroviral therapy was significantly higher compared to female symptomatic HIV infected subjects on 3 months antiretroviral therapy ($p<0.05$). Also,

serum Myoglobin level in male symptomatic HIV infected subjects on 6 months antiretroviral therapy was significantly higher compared to female symptomatic HIV infected subjects on 6 months antiretroviral therapy ($p<0.05$) (see figure 4.22).

Total Creatine kinase:

Pairewise comparisons showed that the serum activity of Total CK in male symptomatic HIV infected subjects before antiretroviral therapy was significantly higher compared to female symptomatic HIV infected subjects before antiretroviral therapy ($p<0.05$) (see figure 4.23).

Creatine kinase MB:

The serum CK-MB level was the same between sex compared with symptomatic HIV infected subjects before antiretroviral therapy and after commencement of therapy followed up for 12 months ($p>0.05$) (see figure 4.24).

Troponin I, Lactate dehydrogenase and CD4 counts in HIV infected subjects before Antiretroviral therapy and after commencement of therapy followed up for 12 months.

Troponin I:

The serum Troponin (ng/ml) level in symptomatic HIV infected subjects before therapy and when on 3, 6, 9 and 12 months therapy were: 1.47±0.21, 1.17±0.62, 1.50±0.55 and 1.28±0.59 respectively. Pairewise comparisons showed that the serum Troponin was significantly higher in symptomatic HIV infected subjects before therapy compared to when on 12 months therapy at $p<0.05$. However, the serum LDH level in symptomatic HIV infected subjects before therapy was the same compared to when on 3, 6 and 9 months therapy at $p>0.05$ respectively. The serum Troponin level in symptomatic HIV infected subjects on 3 months therapy was significantly higher compared to when on 9 and 12 months therapy at $p<0.05$ respectively. However, the serum Troponin level in symptomatic HIV infected subjects on 3 months therapy was the same compared to when on 6 months therapy at $p>0.05$ respectively. Again, the serum Troponin level in symptomatic HIV infected subjects on 6 months therapy was significantly higher compared to when on 12 months therapy at $p<0.05$. Again, the

serum Troponin level in symptomatic HIV infected subjects on 9 months therapy was significantly higher compared to when on 12 months therapy at $p<0.05$ (see figure 4.25).

Lactate dehydrogenase:

The serum activities LDH levels in symptomatic HIV infected subjects before therapy and when on 3, 6, 9 and 12 months therapy were: 184.40±23.68, 121.82±104.92, 58.56±19.89 and 40.75±19.55 respectively. Pairewise comparisons showed that serum activity LDH level in symptomatic HIV infected subjects before therapy was significantly higher compared to when on 3, 6, 9 and 12 months therapy at $p<0.05$ respectively. Similarly, the serum activity LDH level in symptomatic HIV infected subjects at 3 months therapy was significantly higher compared to when on 6, 9 and 12 months therapy at $p<0.05$ respectively, Also, the serum activity LDH level in symptomatic HIV infected subjects on 6 months therapy was significantly higher compared to when on 9 and 12 months therapy at $p<0.05$ respectively. Again, the serum activity LDH level in symptomatic HIV subject on 9 month therapy was significantly higher compared to when on 12 month therapy at $p<0.05$ (see figure 4.26).

CD4 counts:

The blood CD4/µl counts in symptomatic HIV infected subjects before therapy and when on 3, 6, 9 and 12 months therapy were: 362.93±104.56, 251.57±89.50, 354.92±109.95 and 522.32±182.88 respectively. Pairewise comparisons showed that the blood CD4 count was significantly higher in symptomatic HIV infected subjects before therapy compared to when on 12 months therapy at $p<0.05$. But the blood CD4 counts was the same in symptomatic HIV infected subjects before therapy compared to when on 3 months therapy at $p<0.05$. The blood CD4 count was significantly lower in symptomatic HIV infected subjects before therapy compared to when on 9 and 12 months therapy at $p<0.05$ respectively. Again, the blood CD4 count was significantly lower in symptomatic HIV infected subjects on 6 months therapy compared to when on 9 and 12 months therapy at $p<0.05$ respectively, also the blood CD4 count was significantly lower in symptomatic HIV infected subjects on 9 months therapy compared to when on 12 months therapy at $p<0.05$ (see fig 4.27).

Troponin I, Lactate dehydrogenase and CD4 counts studied between sex distributions in symptomatic HIV infected subjects before Antiretroviral therapy and after commencement of therapy followed up for 12 months.

Troponin I level:

The serum Troponin I level in male symptomatic HIV infected subjects on 3 months antiretroviral therapy was significantly higher compared to female symptomatic HIV infected subjects on 3 months antiretroviral therapy ($p<0.05$). Similarly, the serum Troponin I level in male symptomatic HIV infected subjects on 12 months antiretroviral therapy was significantly lower compared to female symptomatic HIV infected subjects on 12 months antiretroviral therapy (see figure 4.28).

Lactate dehydrogenase:

Pairewise comparisons showed that the serum activity of LDH in male symptomatic HIV infected subjects before antiretroviral therapy was significantly lower compared to female symptomatic HIV infected subjects before antiretroviral therapy ($p<0.05$) (see figure 4.29).

CD4 COUNTS:

Pairewise comparisons showed that the blood CD4 count in male symptomatic HIV infected subjects before antiretroviral therapy was significantly lower compared to female symptomatic HIV infected subjects before antiretroviral therapy. However, the blood CD4 count in male symptomatic HIV infected subjects on 12 months antiretroviral therapy was significantly lower compared to female symptomatic HIV infected subjects on 12 months antiretroviral therapy ($p<0.05$) (see figure 4.30).

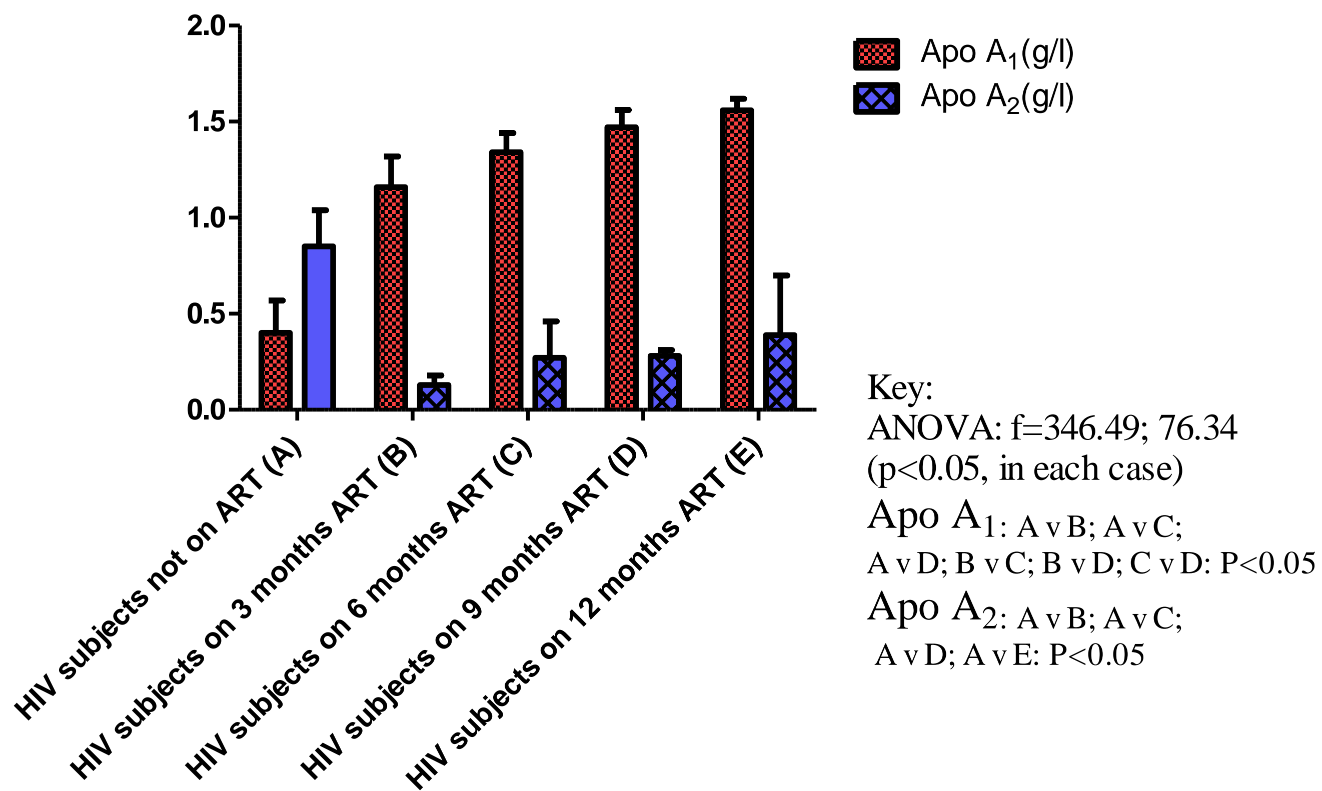


Fig 4.1: Showing a mean ± SD serum levels of Apo A_1 & A_2 in follow-up subjects in pre- and post therapy.

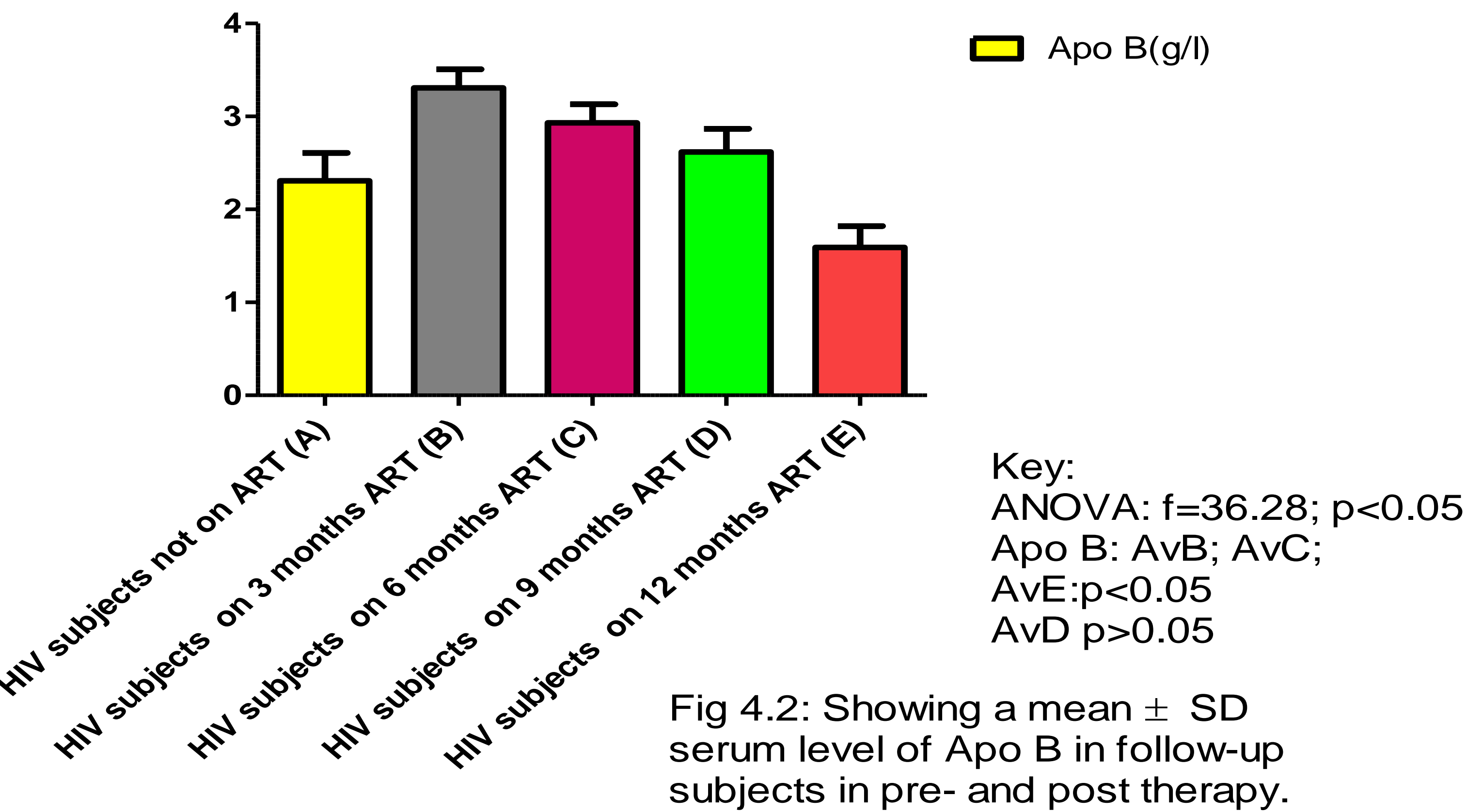


Fig 4.2: Showing a mean ± SD serum level of Apo B in follow-up subjects in pre- and post therapy.

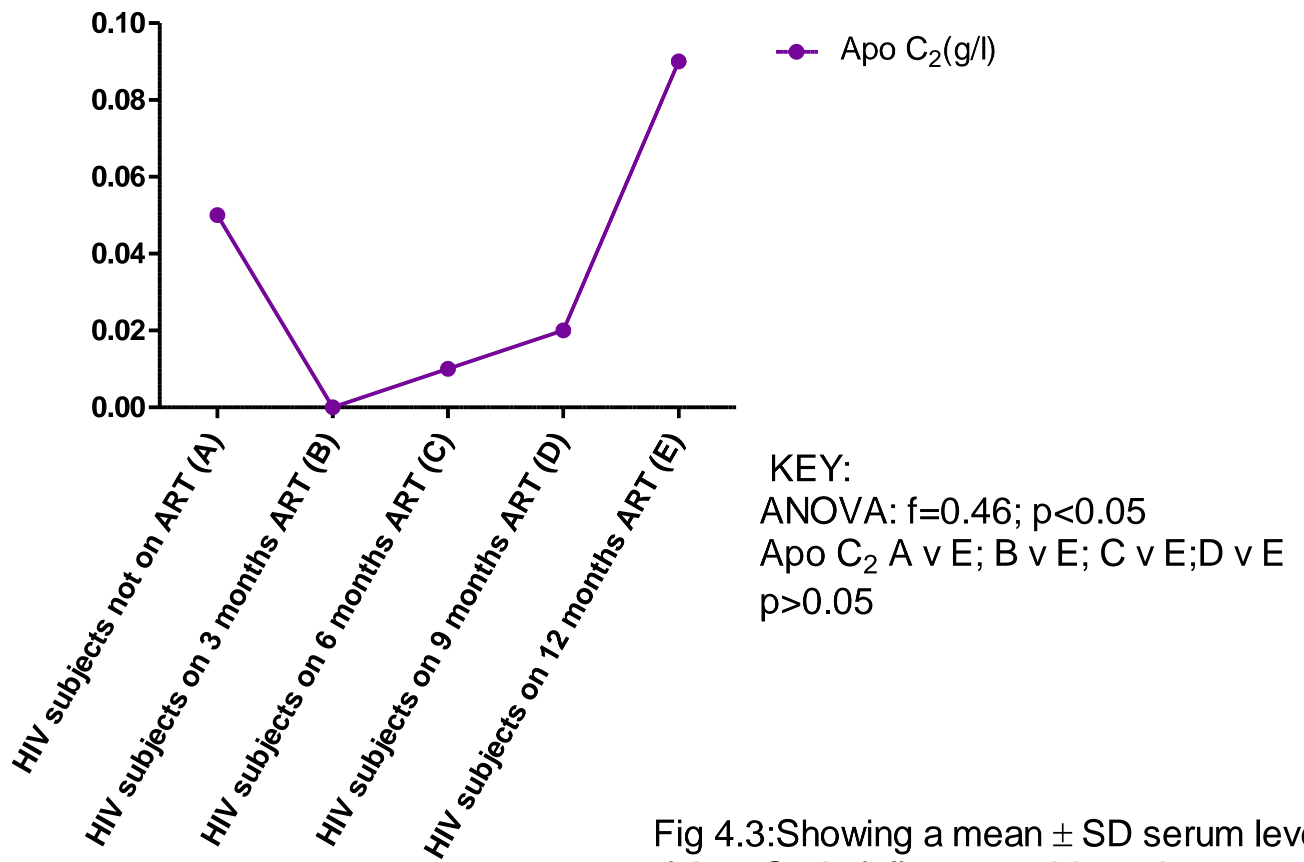
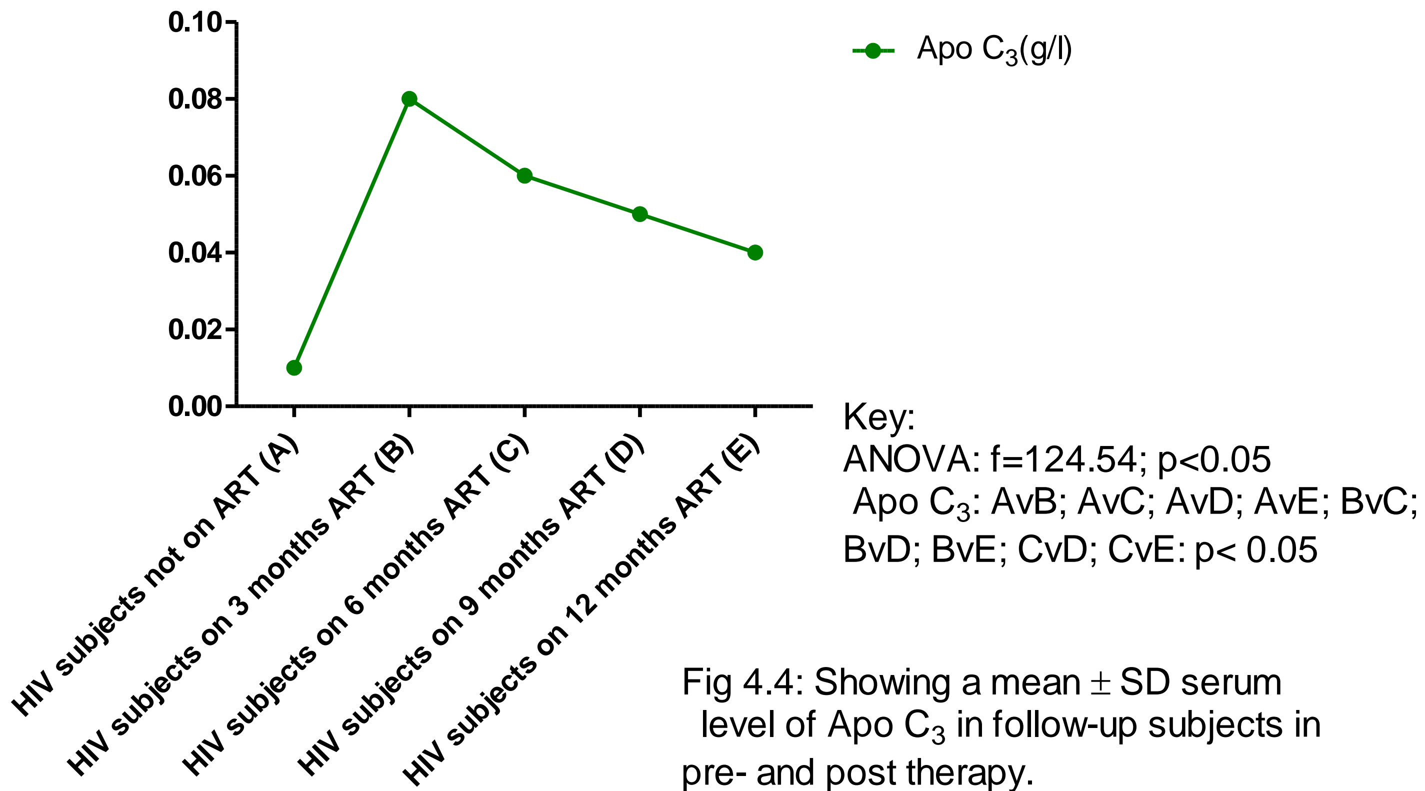


Fig 4.3:Showing a mean ± SD serum level of Apo C_2 in follow-up subjects in pre- and post therapy.



Key:
ANOVA: f=124.54; $p<0.05$
Apo C_3: AvB; AvC; AvD; AvE; BvC; BvD; BvE; CvD; CvE: $p< 0.05$

Fig 4.4: Showing a mean ± SD serum level of Apo C_3 in follow-up subjects in pre- and post therapy.

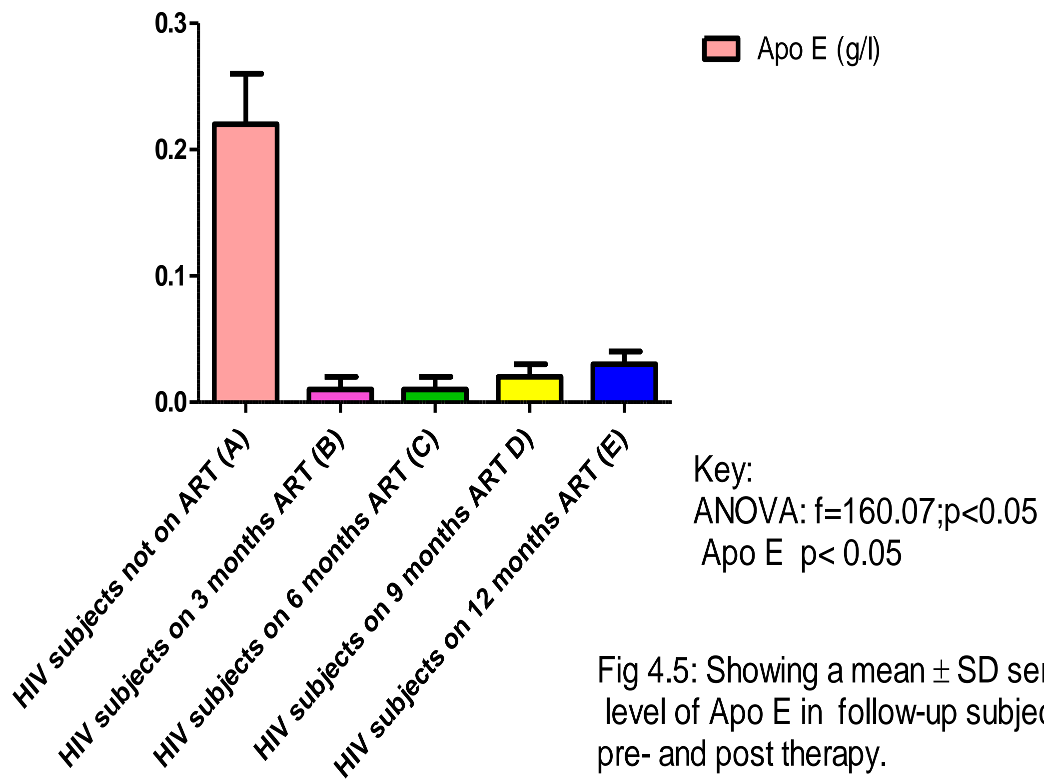


Fig 4.5: Showing a mean ± SD serum level of Apo E in follow-up subjects in pre- and post therapy.

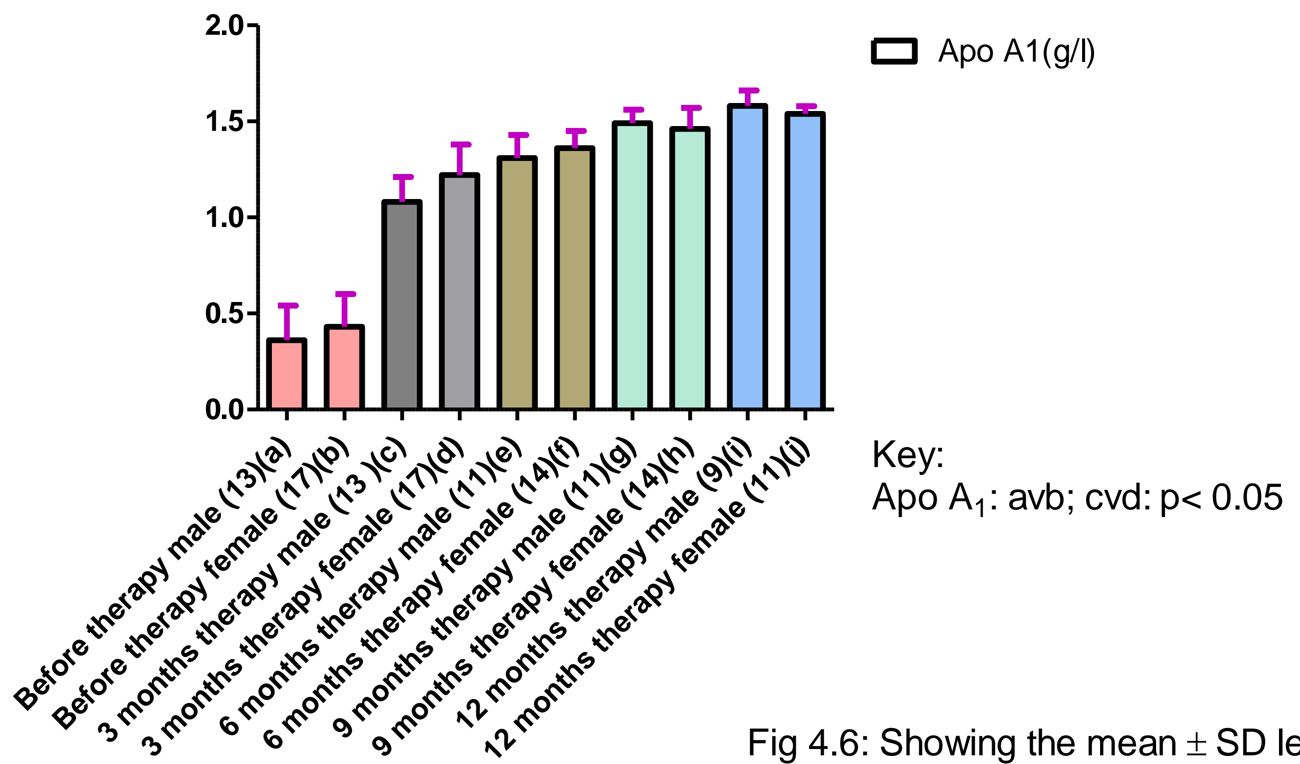


Fig 4.6: Showing the mean ± SD level of Apo A_1 in sex distribution in follow-up subjects in pre- and post therapy.

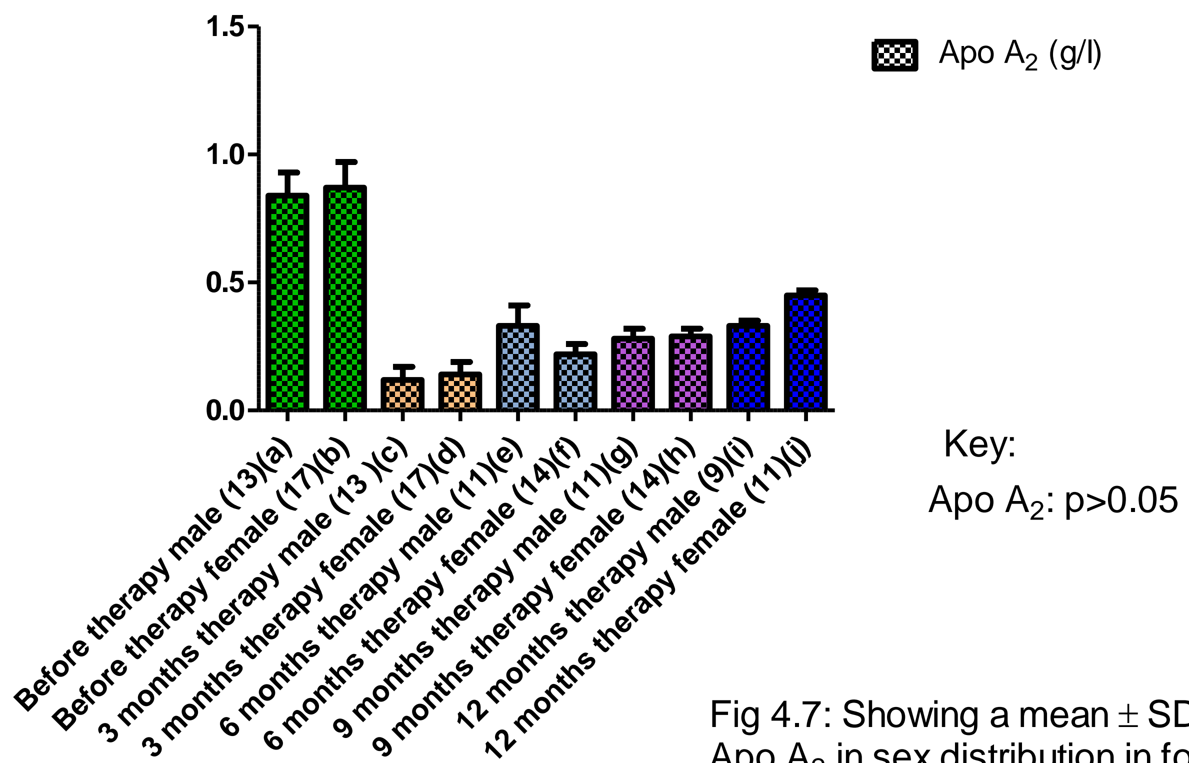


Fig 4.7: Showing a mean ± SD level of Apo A_2 in sex distribution in follow-up subjects in pre- and post therapy.

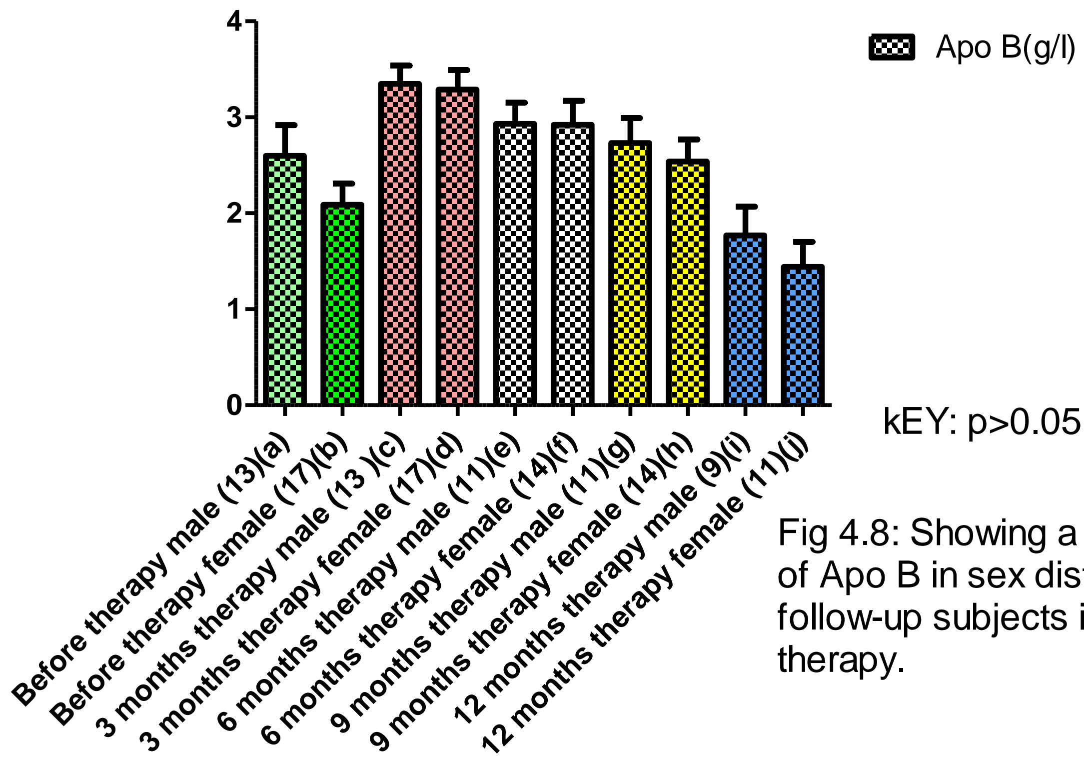


Fig 4.8: Showing a mean ± SD level of Apo B in sex distribution in follow-up subjects in pre- and post therapy.

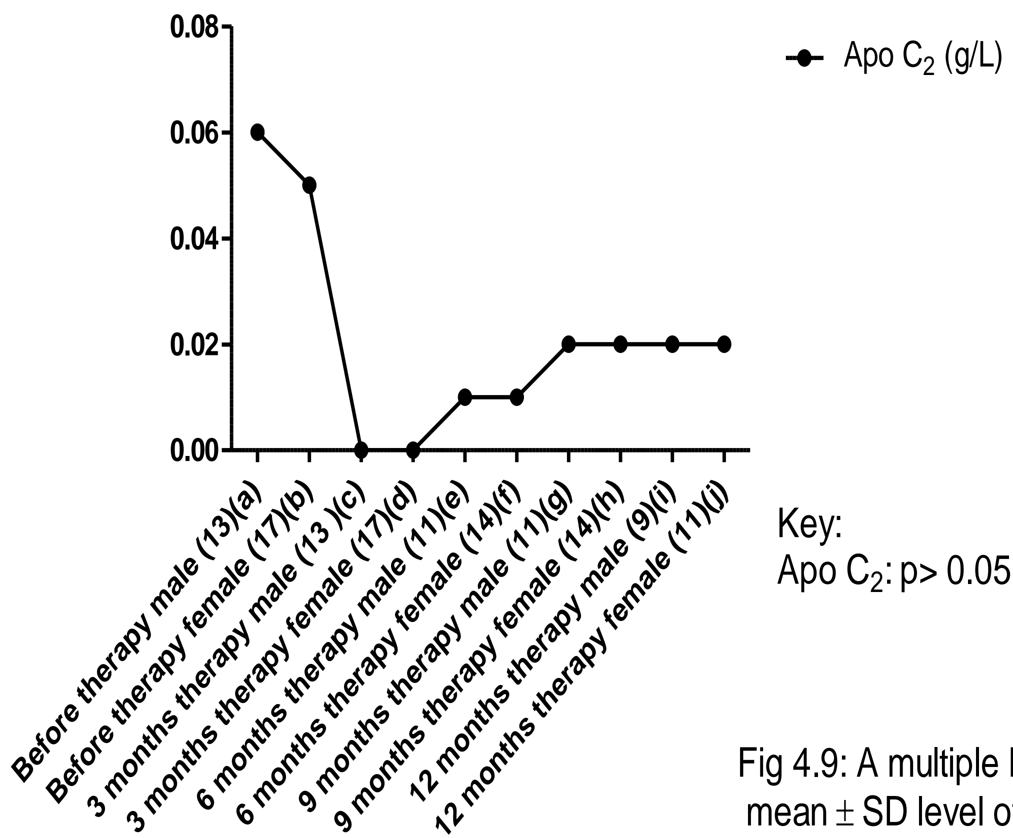
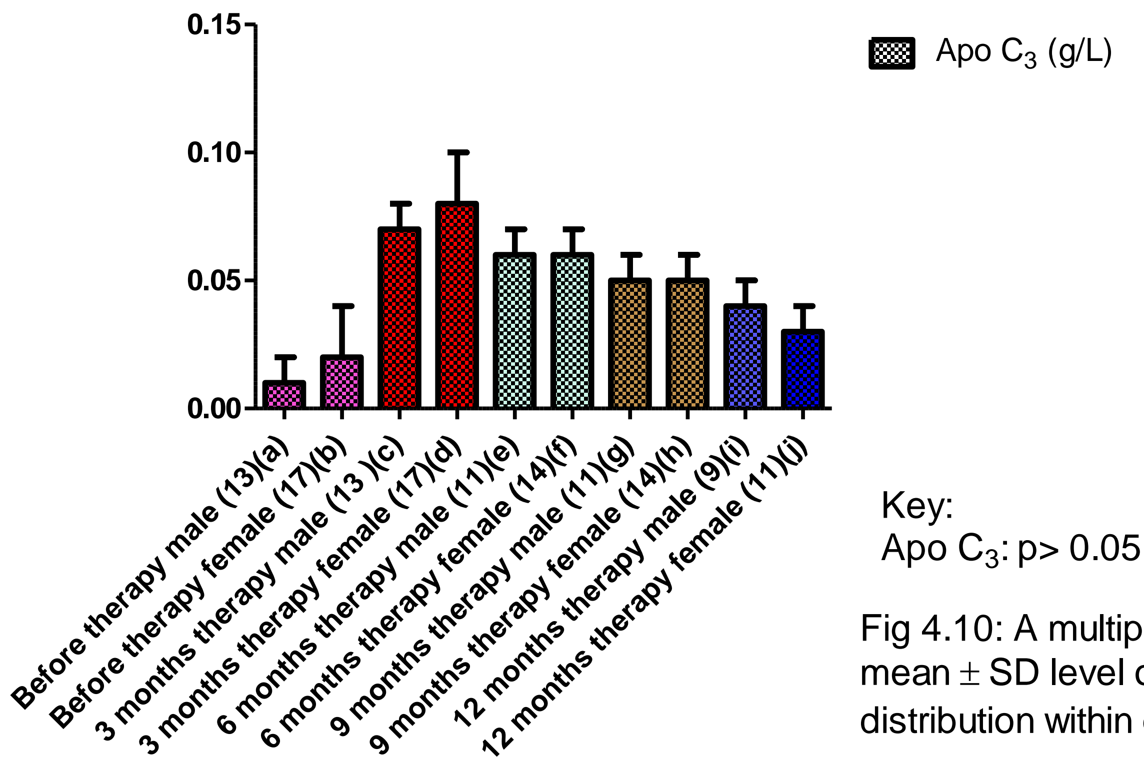
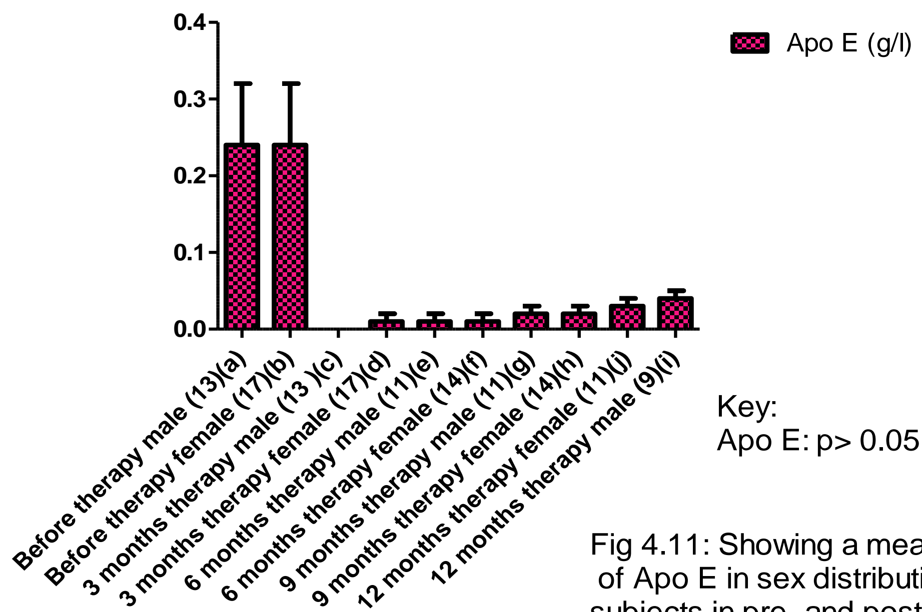


Fig 4.9: A multiple Bar chart showing a mean ± SD level of Apo C_2 in sex distribution within group studied



Key:
Apo C_3: $p > 0.05$

Fig 4.10: A multiple Bar chart showing a mean ± SD level of Apo C_3 in sex distribution within group studied



Key:
Apo E: p> 0.05

Fig 4.11: Showing a mean ± SD level of Apo E in sex distribution in follow-up subjects in pre- and post therapy.

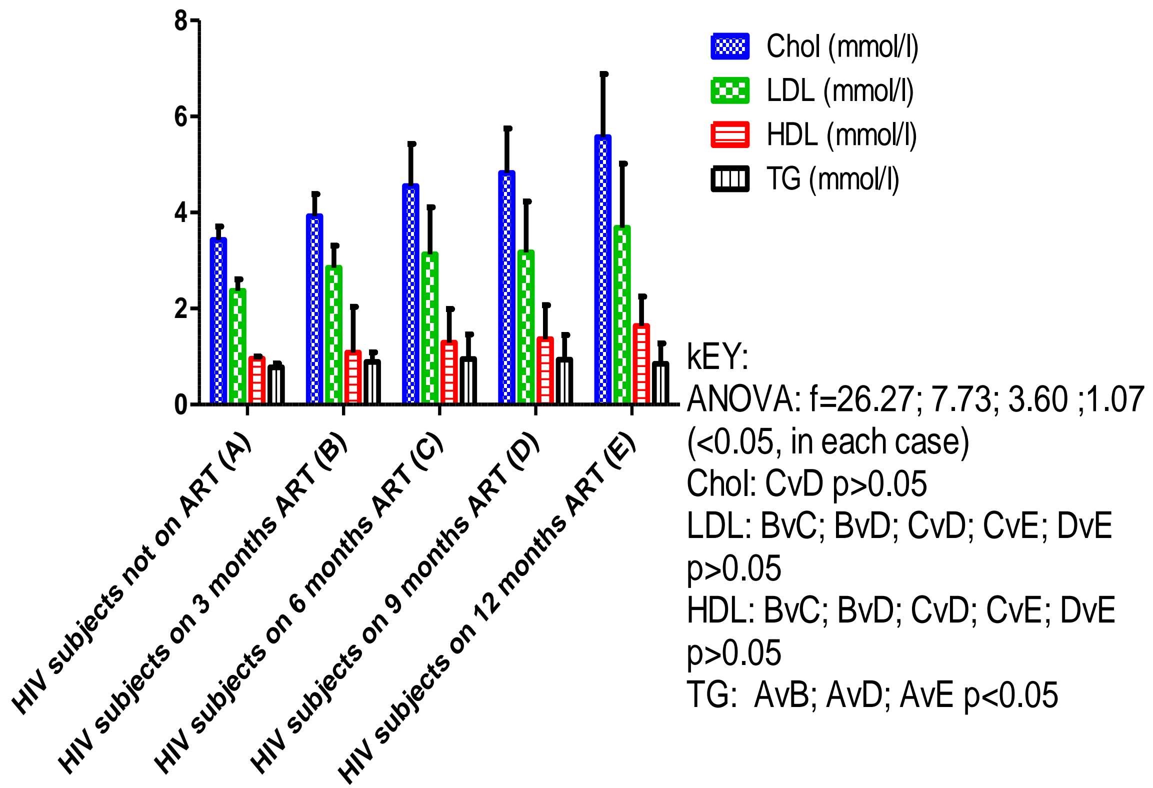


Fig 4.12: Showing a mean ± SD serum levels of lipid profile in follow-up subjects in pre- and post therapy.

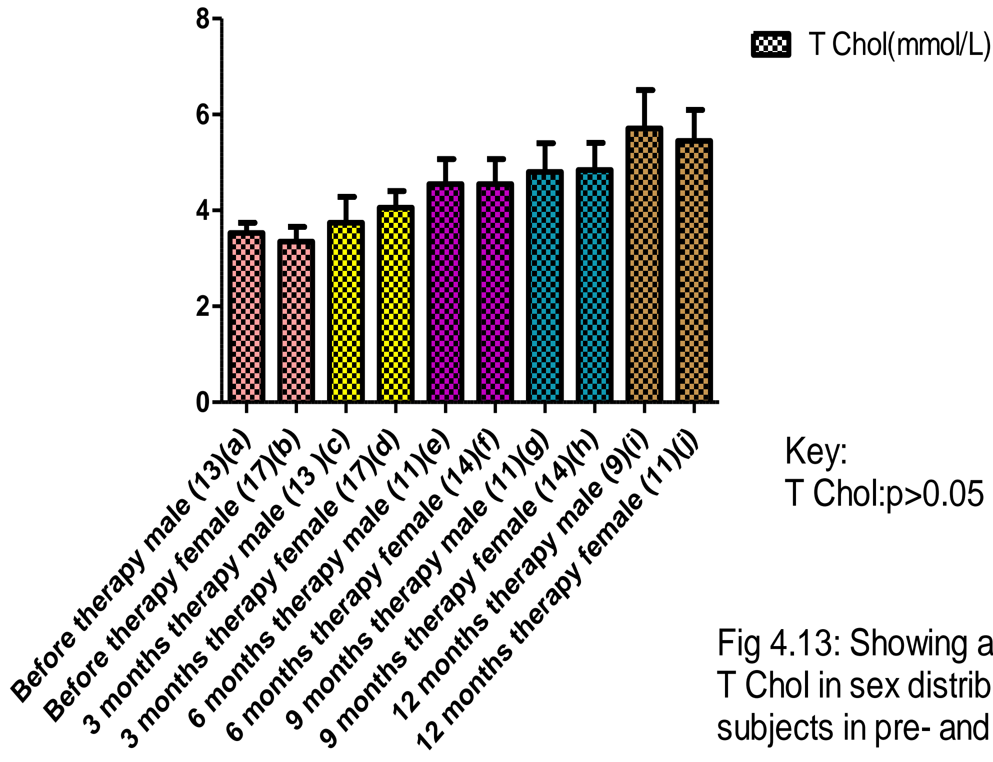


Fig 4.13: Showing a mean ± SD level of T Chol in sex distribution in follow-up subjects in pre- and post therapy.

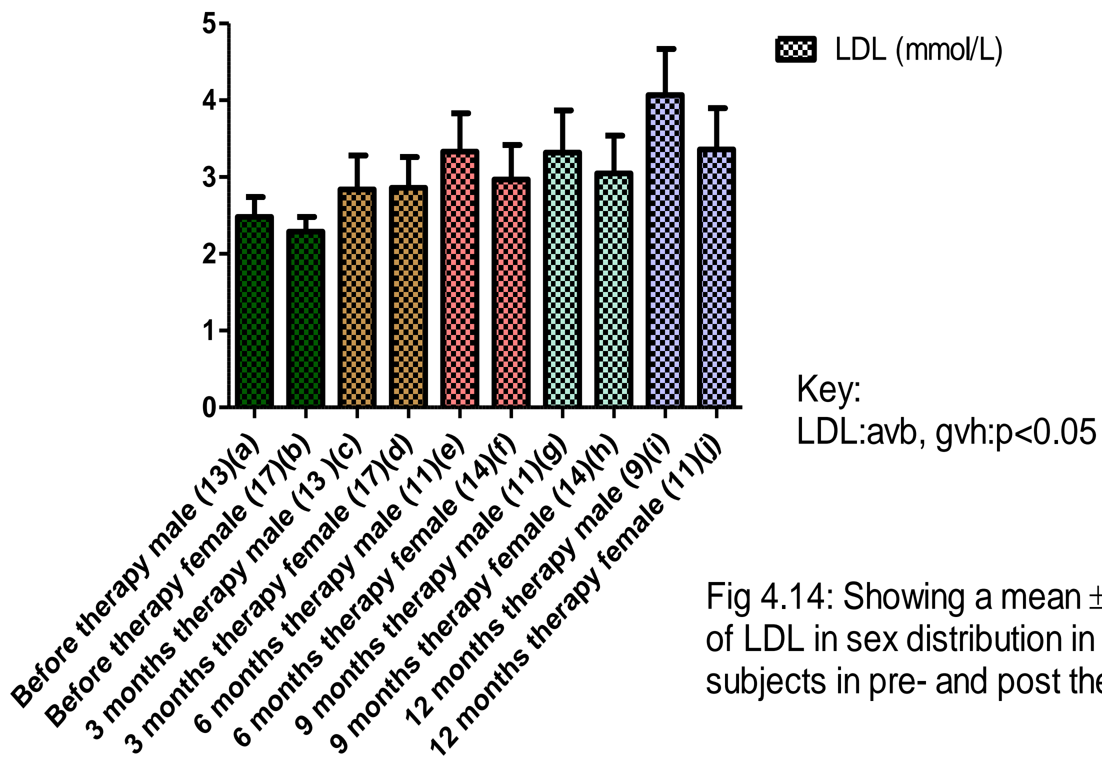


Fig 4.14: Showing a mean ± SD level of LDL in sex distribution in follow-up subjects in pre- and post therapy.

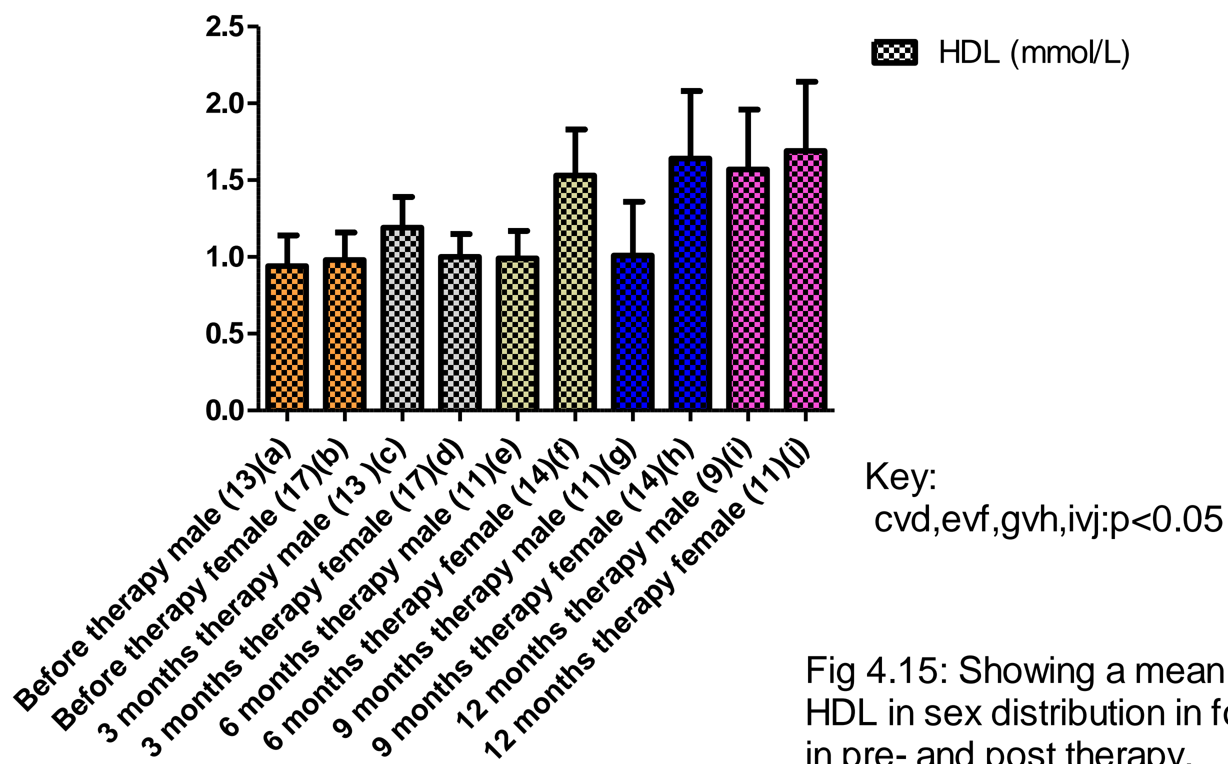


Fig 4.15: Showing a mean ± SD level of HDL in sex distribution in follow-up subjects in pre- and post therapy.

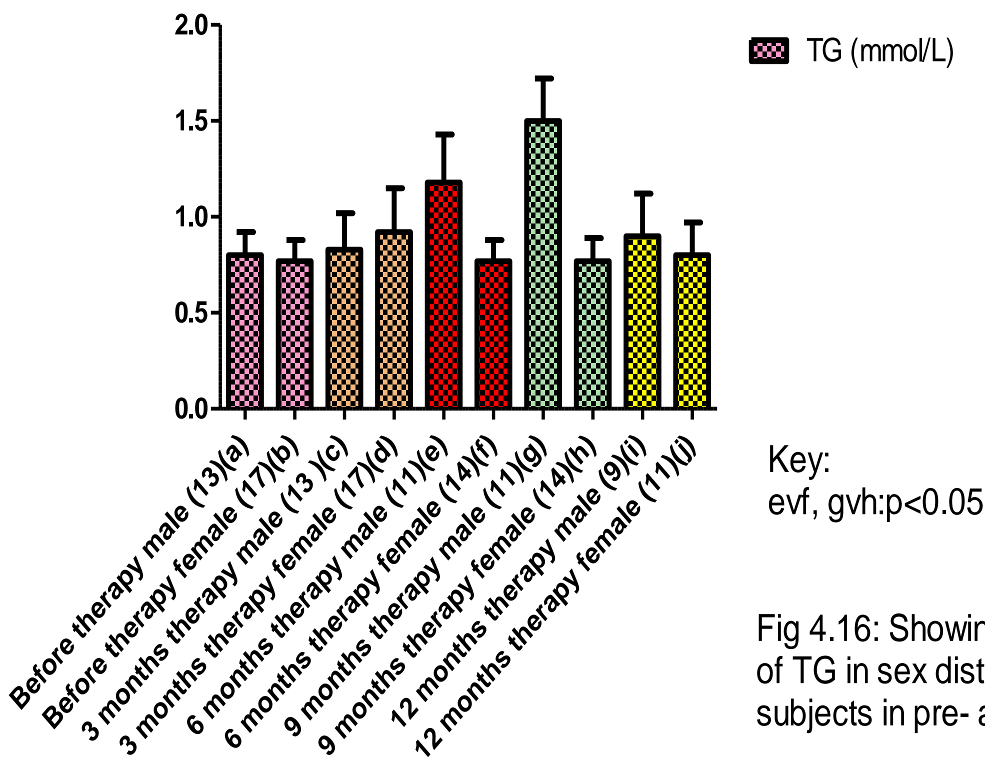


Fig 4.16: Showing a mean ± SD level of TG in sex distribution in follow-up subjects in pre- and post therapy

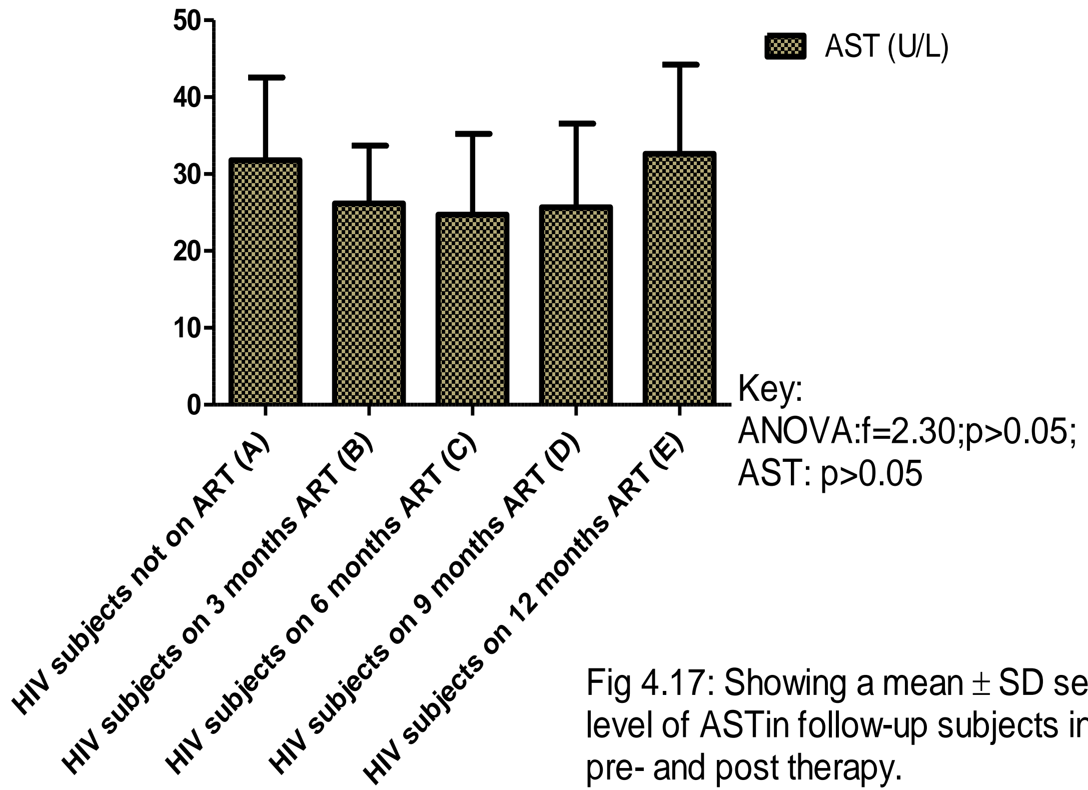


Fig 4.17: Showing a mean ± SD serum level of ASTin follow-up subjects in pre- and post therapy.

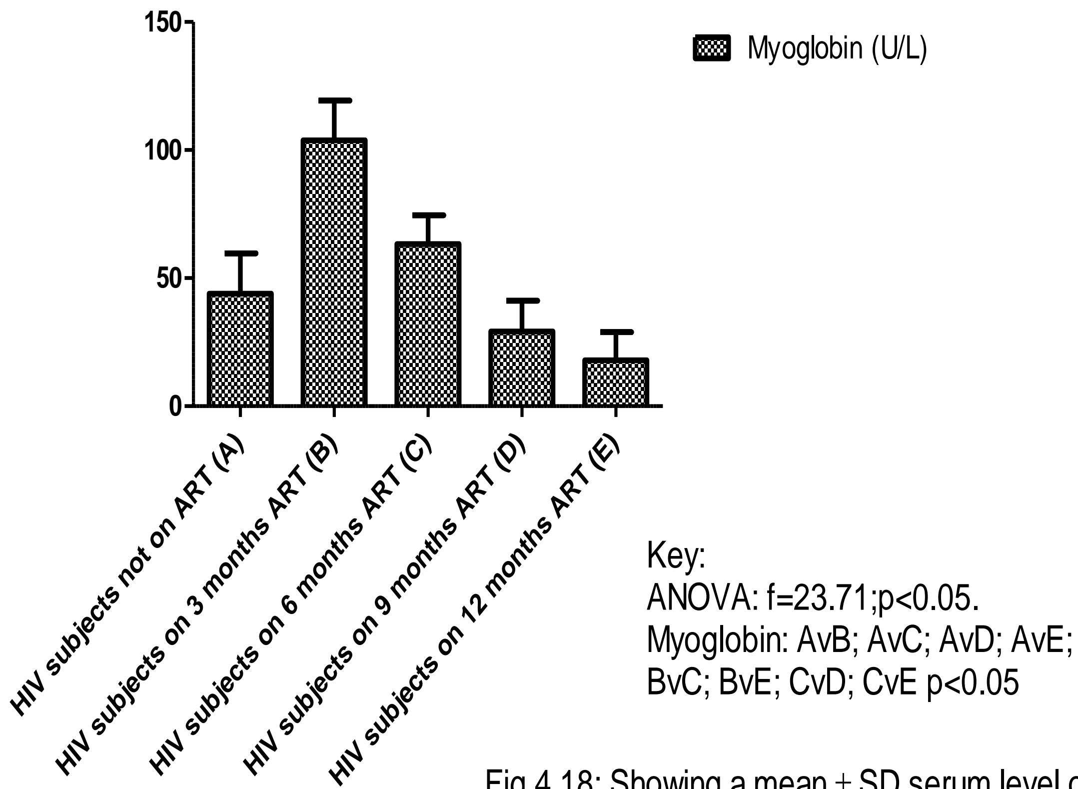


Fig 4.18: Showing a mean ± SD serum level of AST in follow-up subjects in pre- and post therapy.

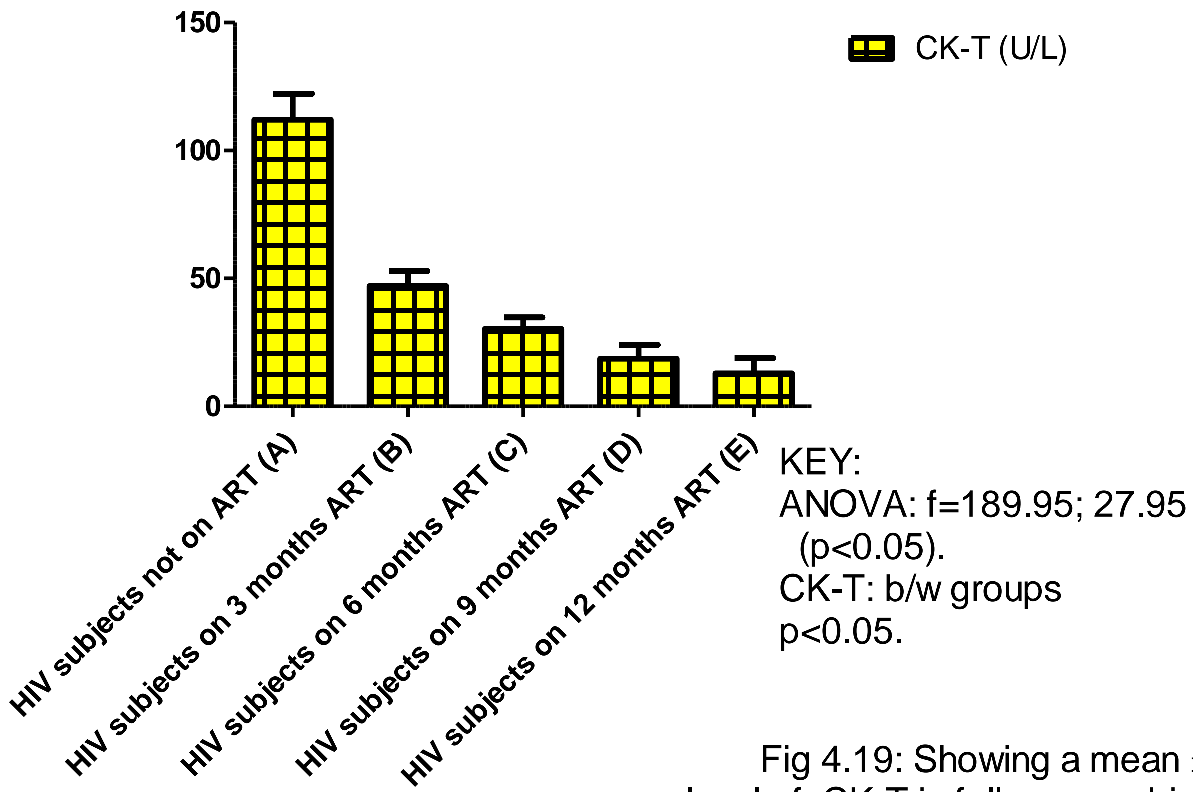


Fig 4.19: Showing a mean ± SD serum level of CK-T in follow-up subjects in pre- and post therapy.

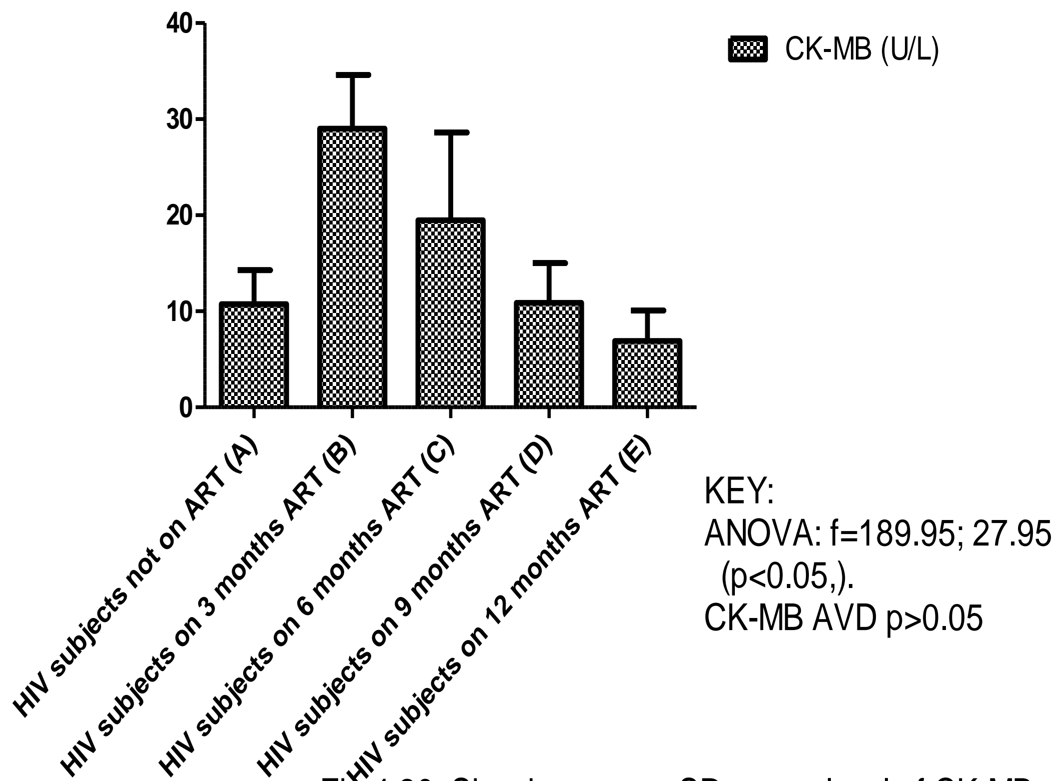


Fig 4.20: Showing a meanSD serum level of CK-MB in follow-up subjects in pre- and post therapy.

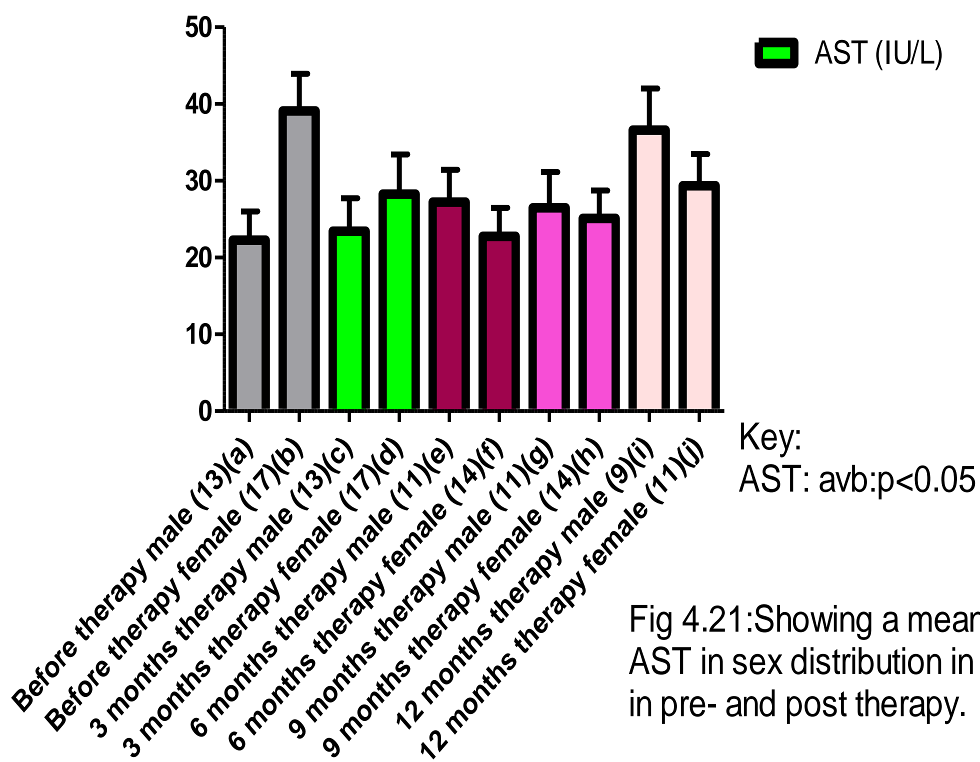


Fig 4.21:Showing a mean ± SD level of AST in sex distribution in follow-up subjects in pre- and post therapy.

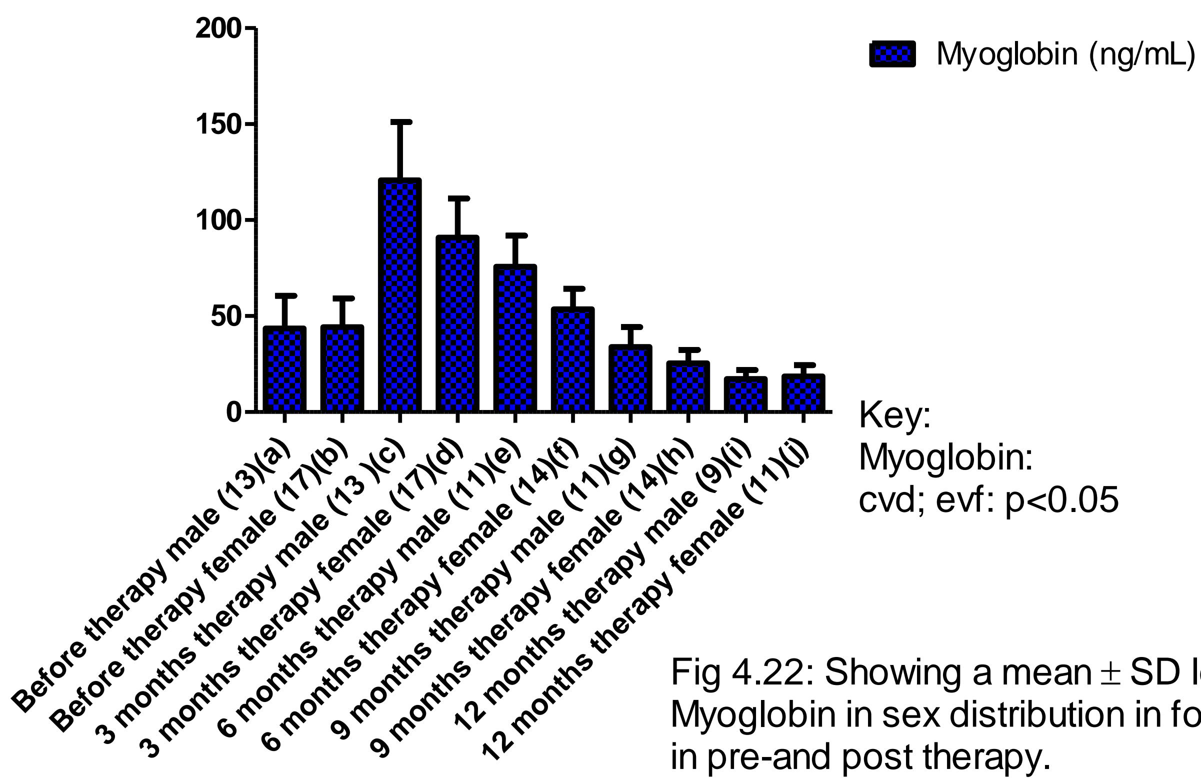


Fig 4.22: Showing a mean ± SD level of Myoglobin in sex distribution in follow-up subjects in pre-and post therapy.

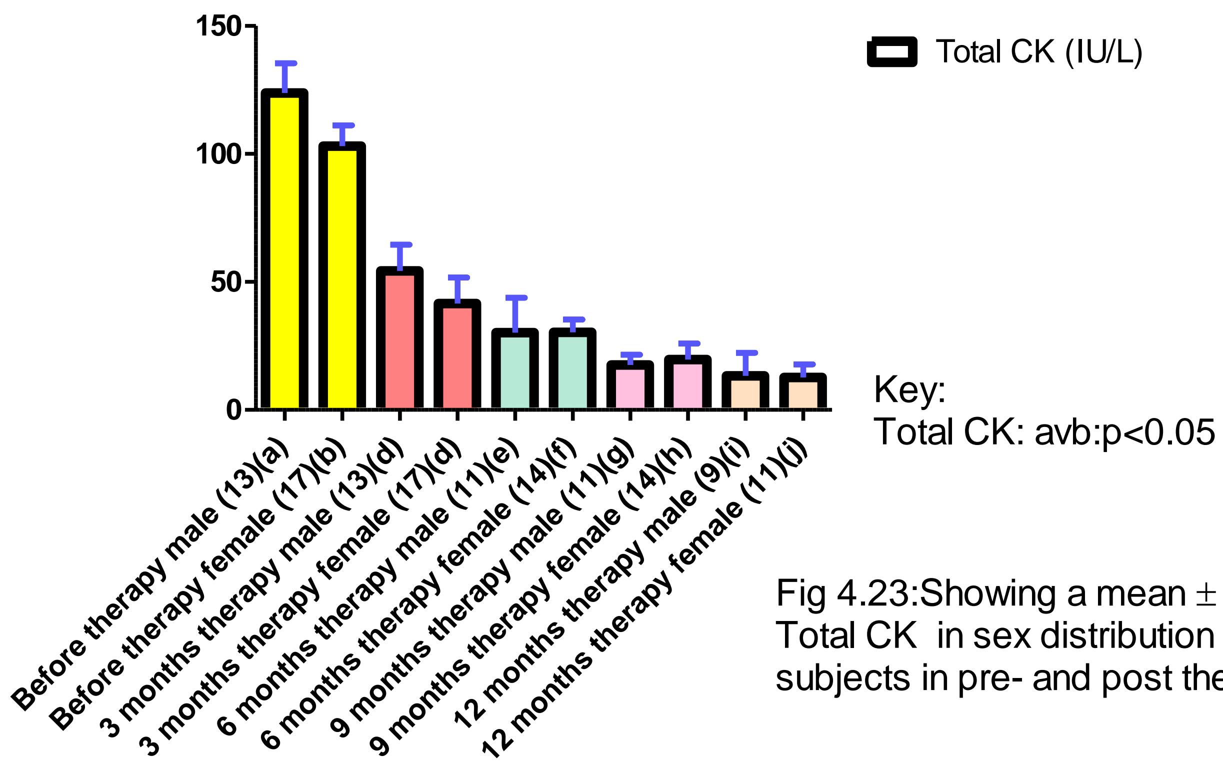


Fig 4.23:Showing a mean ± SD level of Total CK in sex distribution in follow-up subjects in pre- and post therapy.

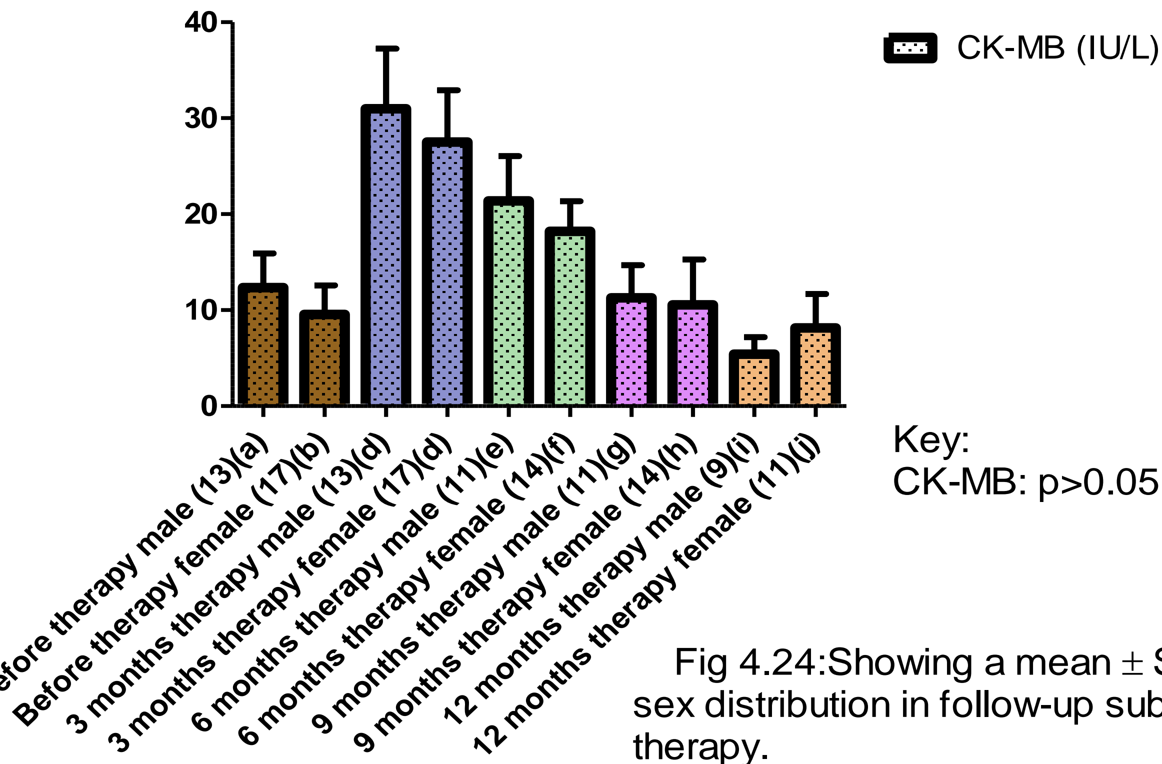


Fig 4.24:Showing a mean ± SD level of CK-MB in sex distribution in follow-up subjects in pre- and post therapy.

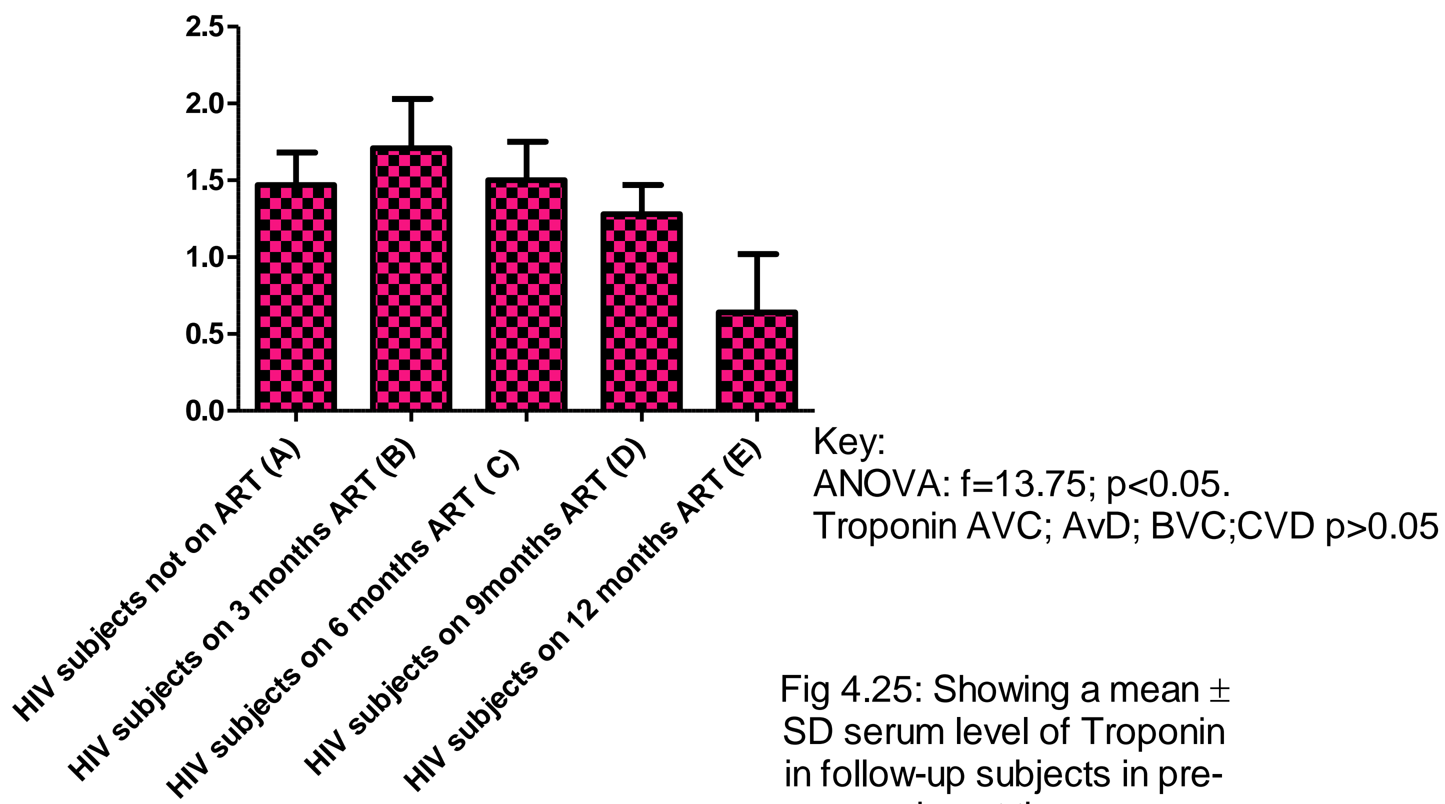


Fig 4.25: Showing a mean ± SD serum level of Troponin in follow-up subjects in pre- and post therapy.

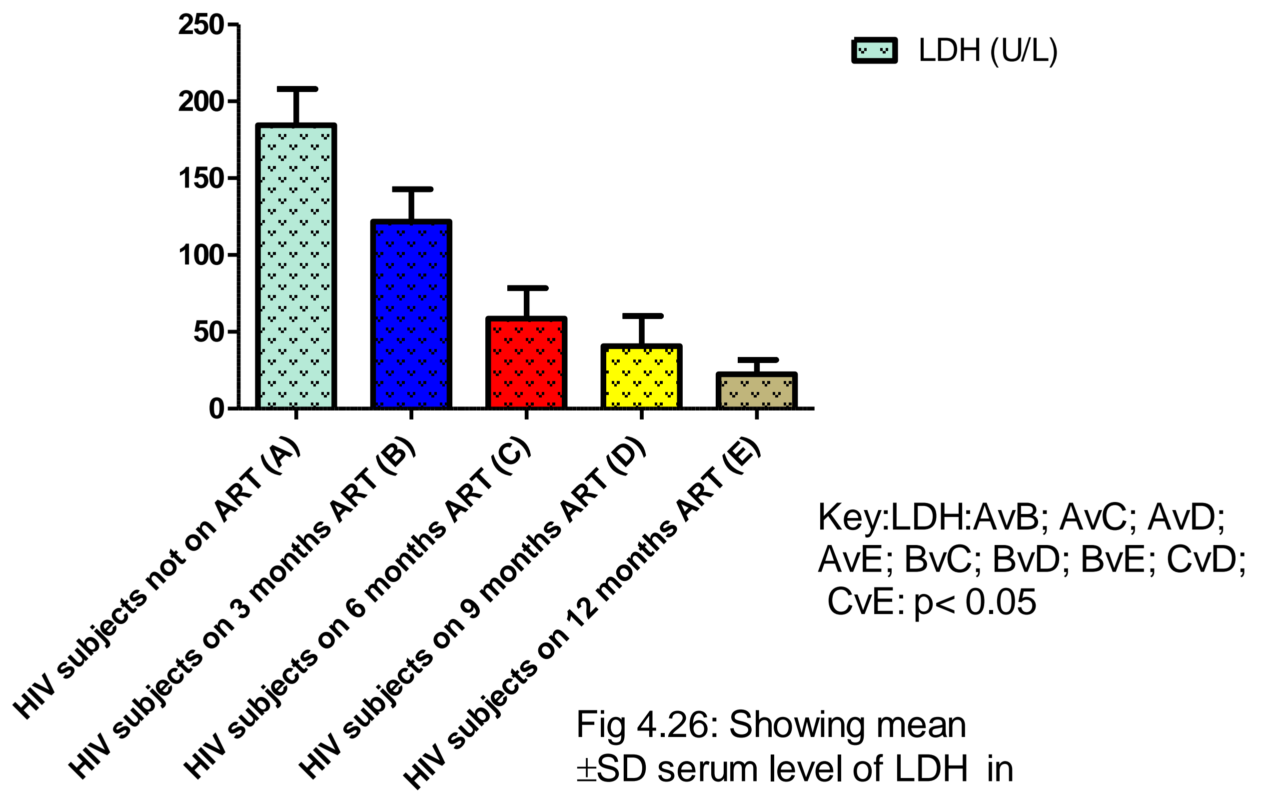


Fig 4.26: Showing mean ±SD serum level of LDH in follow-up subjects in pre- and post therapy.

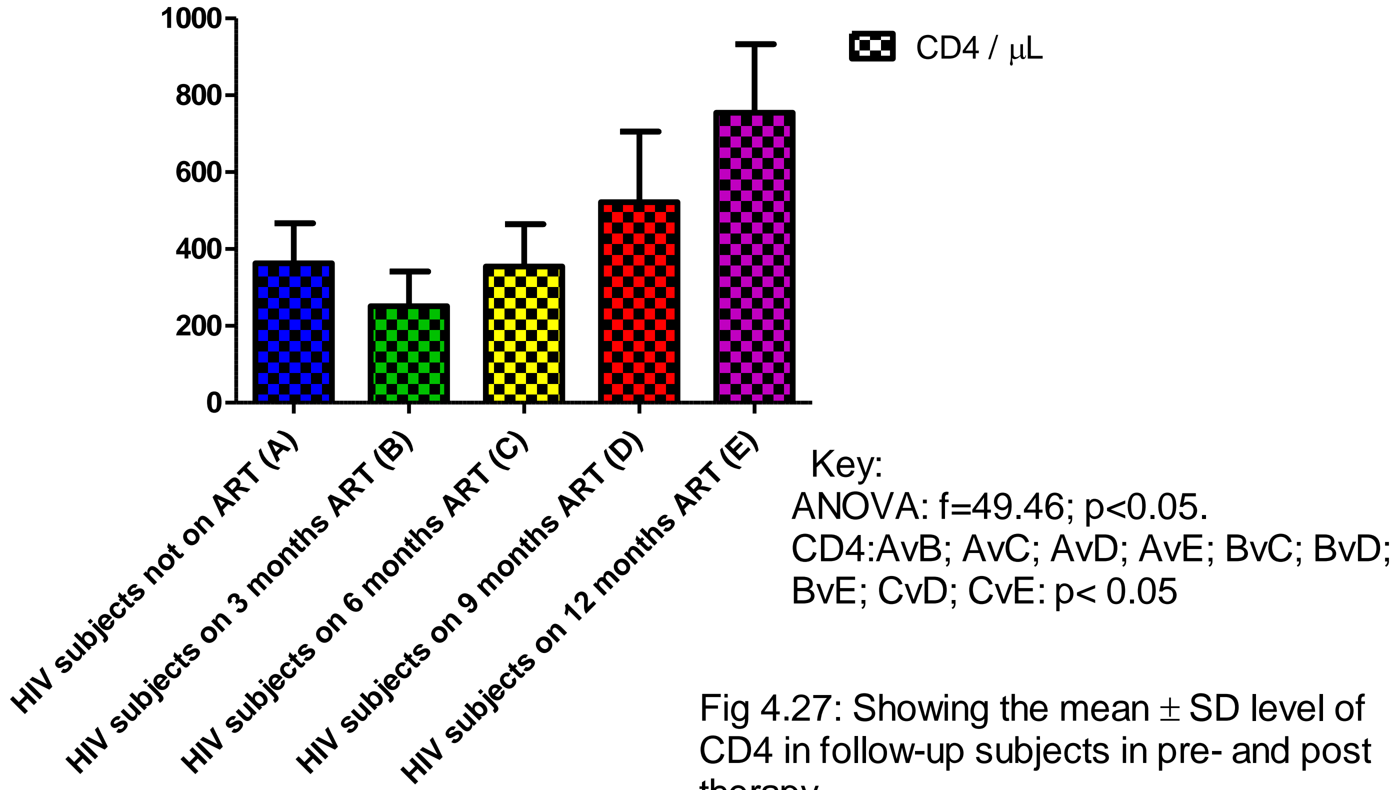


Fig 4.27: Showing the mean ± SD level of CD4 in follow-up subjects in pre- and post therapy.

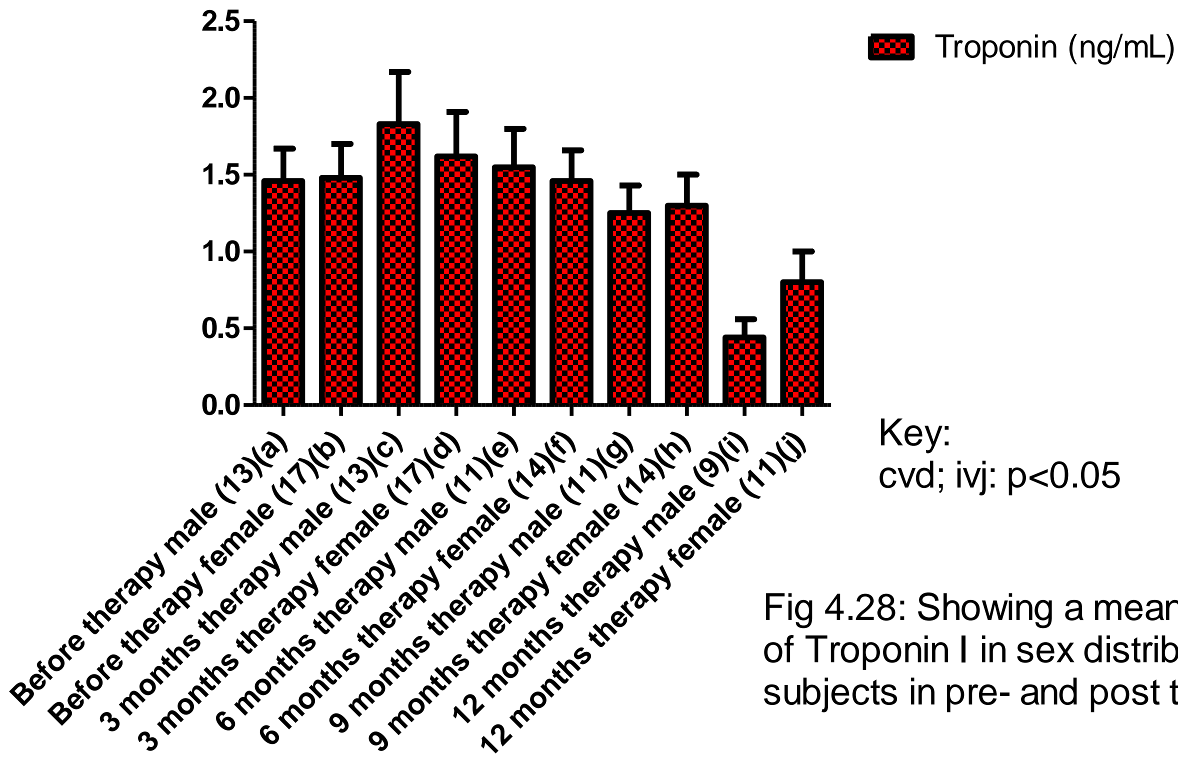


Fig 4.28: Showing a mean ± SD level of Troponin I in sex distribution in follow-up subjects in pre- and post therapy.

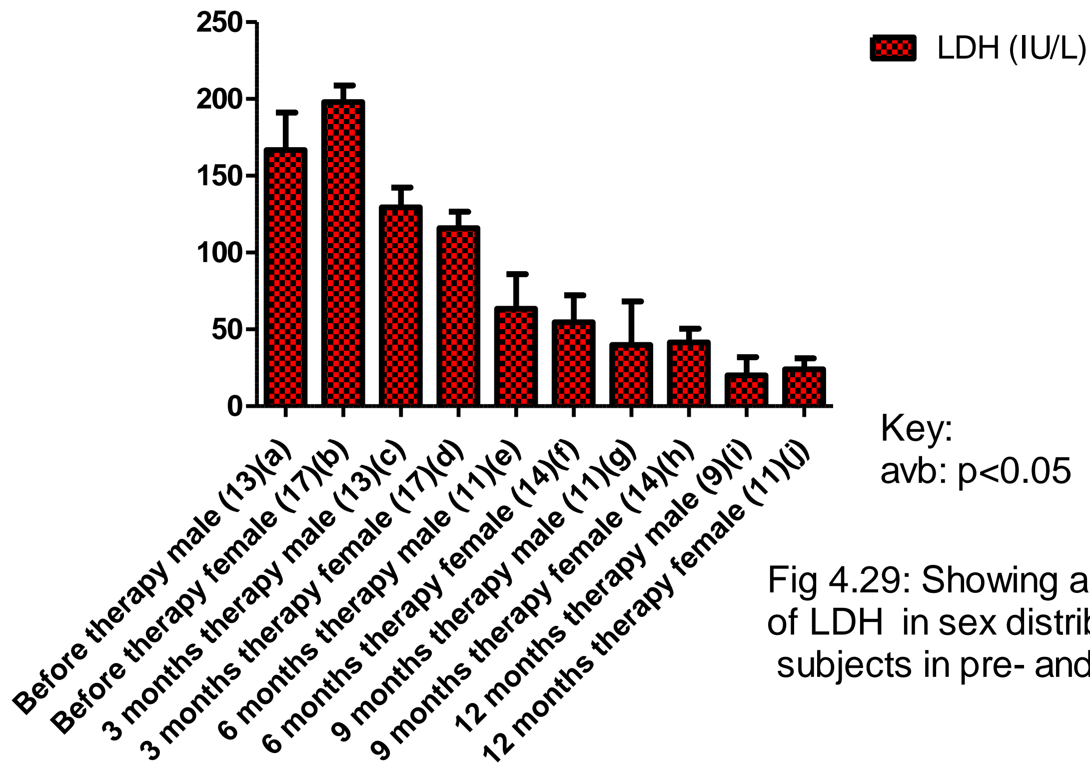


Fig 4.29: Showing a mean ± SD level of LDH in sex distribution in follow-up subjects in pre- and post therapy.

4.5 Apolipoprotein and Lipid profiles in Symptomatic HIV infected subjects before Antiretroviral therapy and after commencement of therapy followed up for 12 months based on 1.5 mg/mL Troponin diagnostic cut-off for myocardial infarction.

4.5.1 Apolipoprotein profile:

The serum Apo A_1, Apo A_2, Apo B, Apo C_2, Apo C_3 and Apo E levels in symptomatic HIV infected subjects before therapy and at 3, 6 and 9 months therapy based on 1.5 mg/ml Troponin diagnostic cut-off for myocardial infarction were significantly different at $P<0.05$ ($F=145.16$, 62.14, 10.89, 40.68, 40.92 and 62.09) respectively. Pairwise comparisons showed that the serum Apo A_1 level was significantly lower in symptomatic HIV infected subjects before therapy compared to when on 3, 6 and 9 months therapy at $P<0.05$ respectively. Similarly, the serum Apo A_1 level was significantly higher in symptomatic HIV infected subjects on 3 months therapy compared to when on 6 and 9 months therapy at $P<0.05$ respectively. Also, the serum Apo A_1 level in symptomatic HIV infected subjects on 6 months therapy was significantly higher compared to when on 9 months therapy at $P<0.05$.

Based on 1.5 mg/ml Troponin diagnostic cut-off for myocardial infarction, pairwise comparisons showed that the serum Apo A_2 level was significantly higher in symptomatic HIV infected subjects before therapy compared to when on 3, 6 and 12 months therapy at $p<0.05$ respectively. Also the serum Apo A_2 level in symptomatic HIV infected subjects on 3 months therapy was significantly higher compared to when on 6 and 9 months $p<0.05$ respectively. Also, the serum Apo A_2 level in symptomatic HIV infected subjects on 6 months was significantly higher compared to 9 months at $p<0.05$ respectively.

Based on 1.5 mg/ml Troponin diagnostic cut-off for myocardial infarction, pairwise comparisons showed that the serum Apo B level was significantly lower in symptomatic HIV infected subjects before therapy compared to when on 3 and 6 months $p<0.05$. But the serum Apo B level was significantly higher in symptomatic HIV infected subjects before therapy compared to when on 9 months at $p<0.05$. The serum Apo B value was significantly higher in symptomatic HIV infected subjects on 3 month therapy compared to when on 6 and 9 months

therapy at $p<0.05$ respectively. But, the serum Apo B value was significantly higher in symptomatic HIV infected subjects on 6 months therapy compared to when on 9 months therapy at $p<0.05$.

Based on 1.5 mg/ml Troponin diagnostic cut-off for myocardial infarction, pairwise comparisons showed that the serum Apo C_2 level was significantly higher in symptomatic HIV infected subjects before therapy compared to when on 3, 6 and 9 months therapy at $p<0.05$ respectively. Also, the serum Apo C_2 level in symptomatic HIV infected subjects on 3 months significantly lower compared to 6 and 9 months $p<0.05$ respectively. Similarly, the serum Apo C_2 level in symptomatic HIV infected subjects on 6 months therapy is significantly lower compared to when on 9 months therapy $p<0.05$.

Based on 1.5 mg/ml Troponin diagnostic cut-off for myocardial infarction, pairwise comparisons showed that the serum Apo C_3 level was significantly lower in symptomatic HIV infected subjects before therapy compared to when on 3, 6 and 9 months therapy at $p<0.05$ respectively. But the serum Apo C_3 level in symptomatic HIV infected subjects on 3 months was significantly higher compared to 6 and 9 months $p<0.05$ respectively. Similarly, the value in symptomatic HIV infected subjects on 6 months therapy was significantly higher compared to symptomatic HIV infected subjects when on 9 months therapy $p<0.05$.

Based on 1.5 mg/ml Troponin diagnostic cut-off for myocardial infarction, pairwise comparisons showed that the serum Apo E level was significantly higher in symptomatic HIV infected subjects before therapy compared to when on 3, 6 and 9 months therapy at $p<0.05$ respectively. But the serum Apo E level in symptomatic HIV infected subjects on 3 months therapy was significantly lower compared to symptomatic HIV infected subjects when on 6 and 9 months $p<0.05$ respectively. Also, the serum Apo E level in symptomatic HIV infected subjects on 6 months therapy was significantly higher compared to symptomatic HIV infected subjects when on 9 months therapy at $p<0.05$.

4.5.2 Lipid profile:

The serum Total Cholesterol, LDL and HDL levels in symptomatic HIV infected subjects before and on 3, 6 and 9 months therapy were significantly different at $p<0.05$ ($F=21.40, 7.18$ and 8.21)respectively. However the serum TG level was not significantly different at $p>0.05$($F=0.77$). Based on 1.5 mg/ml Troponin diagnostic cut-off for myocardial infarction, pairwise comparisons showed that the serum total Cholesterol level was significantly lower in symptomatic HIV infected subjects before therapy compared to when on 3, 6 and 9 months therapy at $p<0.05$ respectively. Similarly, the serum total Cholesterol level in symptomatic HIV infected subjects on 3 months therapy compared to when on 6 and 9 months therapy at $p<0.05$ respectively. Also, the serum total Cholesterol level was significantly lower in symptomatic HIV infected subjects on 6 months therapy compared to when on 9 months therapy at $p<0.05$. Again, based on 1.5 mg/ml Troponin diagnostic cut-off for myocardial infarction, pairwise comparisons showed that the serum LDL level was significantly lower in symptomatic HIV infected subjects before therapy compared to when on 3 and 9 months therapy at $p<0.05$ respectively. But the serum LDL in symptomatic HIV subjects before therapy was significantly higher compared to when on 6 months therapy at $p<0.05$ respectively. The serum LDL was significantly higher in symptomatic HIV infected subjects on 3 months therapy compared to when on 6 months therapy at $p<0.05$. But the serum LDL level in symptomatic HIV infected subjects on 3 months was significantly lower compared to when on 9 months therapy at $p<0.05$. However, the serum LDL level was the same in symptomatic HIV infected subjects on 6 months therapy compared to when on 9 months therapy at $p>0.05$.

Also, based on 1.5 mg/ml Troponin diagnostic cut-off for myocardial infarction, between group comparison showed that the serum HDL level was significantly lower in symptomatic HIV infected subjects before therapy compared to when on 9 months therapy at $p<0.05$. Similarly, the serum HDL was significantly lower compared to when on 6 and 9 months $p<0.05$ respectively. Also the mean serum HDL level in symptomatic HIV infected subjects

on 6 months therapy was significantly lower in symptomatic HIV infected subjects on 3 months therapy compared to when on 9 months therapy p<0.05. Other comparisons showed the same value at p>0.05 respectively.

Similarly, based on 1.5 mg/ml Troponin diagnostic cut-off for myocardial infarction, Pairwise comparisons showed that the serum TG level was significantly lower in symptomatic HIV infected subjects before therapy compared to when on 3 months therapy at p<0.05. Other comparisons showed the same value at p>0.05 respectively (see table 4.5).

4.6 Cardiac markers and CD4 count in symptomatic HIV infected subjects before Antiretroviral therapy and after commencement of therapy followed up for 12 months based on 1.5 mg/mL Troponin diagnostic cut-off for myocardial infarction.

The serum Myoglobin, Troponin (ng/mL) and serum activities of Total CK , CK MB, LDH(U/L) and CD4 (/µL) were significant at P< 0.05 (F= 3.47, 6.74, 107.92, 8.21, 11.27 and 11.03) respectively. However, the serum activity of AST was not significant at P > 0.05 (0.77). Pairwise comparisons showed that the serum activity of AST was significantly lower in symptomatic HIV infected subjects before therapy compared to when on 3 and 6 months therapy at P < 0.05 respectively. But the level of serum Myoglobin level in HIV infected subjects before therapy was the same compared to when on 9 months therapy at P> 0.05. The serum level of Myoglobin in symptomatic HIV infected subjects on 3 months was the same compared to 6 and 9 months therapy at P > 0.05 respectively. However, the serum Myoglobin level in symptomatic HIV infected subjects in 6 months therapy was significantly higher compared to when on 9 months therapy at P < 0.05.

Also pairwise comparisons showed that the serum Troponin level was significantly lower in symptomatic HIV infected subjects before therapy compared to when on 3, 6, and 9 months therapy at P < 0.05 respectively. Other group comparisons showed no significant differences at P> 0.05 respectively.

Again pairwise comparisons showed that the serum activity of Total CK was significantly higher in symptomatic HIV infected subjects before therapy compared to when on 3, 6 and 9

months therapy $P < 0.05$ in each case. Also the serum activity of Total CK was significantly higher in symptomatic HIV infected subjects on 3 months therapy compared to when on 9 months therapy at $P < 0.05$. But the serum Total CK activity was the same in symptomatic HIV infected subjects on 3 months therapy compared to when on 9 month therapy at $P > 0.05$.

Pairwise comparisons showed that the serum activity of CK-MB was significantly lower in symptomatic HIV infected subjects before therapy compared to when on 3 and 6 months therapy at $P < 0.05$ respectively. Other group comparisons showed no significant differences at $P > 0.05$.

Pairwise comparisons showed that the serum LDH activity in symptomatic HIV infected subjects before therapy was significantly higher compared to when on 3, 6 and 9 months therapy at $P < 0.05$ respectively. Other comparisons showed the same value at $P > 0.05$ respectively.

Again pairwise comparisons showed that the serum AST activity in symptomatic HIV infected subjects before therapy was significantly higher compared to when on 9 months therapy at $P < 0.05$. Other comparisons showed the same value at $P > 0.05$ respectively.

Pairwise comparisons showed that the blood CD4 count was significantly higher in symptomatic HIV infected subjects before therapy compared to when on 3 and 6 months therapy at $P < 0.05$ respectively. But the blood CD4 count in symptomatic HIV infected subjects before therapy was significantly lower compared to when on 9 months therapy at $P < 0.05$. Also, the blood CD4 count in symptomatic HIV infected subjects on 3 months therapy was significantly lower compared to when on 9 months therapy at $P < 0.05$. Similarly, the blood CD4 count in symptomatic HIV infected subjects on 6 months was significantly lower compared to when on 9 months therapy at $P < 0.05$. But the blood CD4 counts in symptomatic HIV infected subjects before therapy and on three months therapy were the same compared to when on 6 months therapy at $P > 0.05$ respectively (see table 4.6).

Table 4.5: Mean ± SD serum levels of Apolipoprotein and lipid profiles in Symptomatic HIV subjects before and after 12 months therapy using 1.5 ng/mL Troponin diagnostic cut-off for myocardial infarction.

Group	Apo A-1(g/L)	Apo A-11 (g/L)	Apo B(g/L)	Apo C-11(g/L)	Apo C-111 (g/L)	Apo E (g/L)	T Chol (mmol/L)	LDL (mmol/L)	HDL (mmol/L)	TG (mmol/L)	TG (mmol/L)
Before therapy (n=20)(A)	0.42 ± 0.13	0.83 ± 0.21	2.30 ± 0.71	0.05 ±0.02	0.01 ± 0.01	0.23 ± 0.07	3.39±0.28	2.35±0.18	0.97±0.04	0.79±0.07	1.75±0.04
3 months therapy (n=15)(B)	1.20 ± 0.15	0.13 ± 0.05	3.36 ± 0.33	0.00 ± 0.00	0.07 ± 0.02	0.00 ± 0.00	4.09±0.29	2.91±0.43	0.98±0.24	0.94±0.17	0.79±0.04
6 months therapy (n=11)(C)	1.31 ± 0.12	0.25 ± 0.05	3.01 ± 0.28	0.01 ± 0.00	0.06 ± 0.01	0.01 ± 0.01	4.45±0.49	2.20±0.63	1.04±0.54	1.00 ±0.70	1.35±0.04
9 months therapy (n=4)(D)	1.54 ± 0.05	0.29 ± 0.04	2.21 ± 0.74	0.02 ± 0.01	0.04 ± 0.02	0.05 ± 0.01	4.98±1.30	3.16±1.32	1.65±0.40	0.83± 0.33	1.44±0.05
F-value	145.16	62.14	10.89	40.68	40.92	62.09	21.40	7.18	8.21	0.77	
p-value	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	>0.05	912.00 (.000)
A v B	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	>0.05	>0.05	<0.05
A v C	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	>0.05	<0.05	>0.05	>0.05	<0.05
A v D	<0.05	<0.05	>0.05	<0.05	<0.05	<0.05	>0.05	<0.05	>0.05	>0.05	<0.05
B v C	<0.05	<0.05	<0.05	>0.05	>0.05	>0.05	>0.05	<0.05	>0.05	>0.05	<0.05
B v D	<0.05	<0.05	<0.05	>0.05	<0.05	<0.05	<0.05	<0.05	<0.05	>0.05	<0.05
C v D	<0.05	>0.05	<0.05	>0.05	<0.05	<0.05	>0.05	>0.05	>0.05	>0.05	<0.05

Table 4.6: Mean ± SD serum levels of Cardiac markers and CD4 in Symptomatic HIV subjects before and after 12 months therapy using 1.5 ng/mL Troponin diagnostic cut-off for myocardial infarction..

Group(n=100)	Myoglobin (ng/mL)	Troponin (ng/mL)	CK-T(IU/L)	CK-MB (IU/L) AST (IU/L)	LDH (IU/L)	CD4 (/µL)	Apo E (g/L)	T Chol (mmol/L)	CD4 (/mL)
Before therapy (n=20)(A)	46.39 ± 16.36	1.59 ± 0.06	109.70 ± 14.10	10.03 ± 2.75 32.87 ± 10.69	185.50 ± 25.31	367.50 ± 96.56	0.10 ± 0.06	5.72±0.43	
3 months therapy (n=15)(B)	87.53 ± 62.70	2.00 ± 0.38	38.71 ± 15.80	25.01 ± 12.79 26.89 ± 8.22	98.02 ± 10.64	285.47 ± 112.82	0.24 ± 0.11	3.51±0.22	619.58 ± 392.74
6 months therapy (n=11)(C)	70.35 ± 27.38	1.95 ± 0.23	28.70 ± 7.85	17.99 ± 5.65 25.92 ± 10.39	58.42 ± 27.00	350.00 ± 99.40	0.11 ± 0.07	4.27±0.15	374.78 ± 121.59
9 months therapy (n=4)(D)	31.00 ± 14.98	2.13 ± 0.53	19.95 ± 5.90	12.93± 4.17 19.23 ± 5.37	31.85 ± 17.08	594.00 ± 151.70	0.05 ± 0.01	4.62±0.24	437.20 ±129.75
					11.27				
				8.21					
				0.77					
F-value	3.47	6.74	107.92	<0.05	<0.05	11.03	131.12	107.10	940.64 ±
p-value	<0.05	<0.05	<0.05	>0.05		<0.05	0.000	0.000	148.85
				<0.05	<0.05				123.10
A v B	<0.05	<0.05	<0.05	>0.05		<0.05	<0.05	<0.05	(.000)
				<0.05	<0.05				
A v C	<0.05	<0.05	<0.05	>0.05		>0.05	>0.05	<0.05	<0.05
				>0.05	<0.05				
A v D	>0.05	<0.05	<0.05	>0.05		<0.05	<0.05	<0.05	>0.05
				>0.05	>0.05				
B v C	>0.05	>0.05	>0.05	>0.05		>0.05	<0.05	<0.05	<0.05
				>0.05	>0.05				
B v D	>0.05	>0.05	<0.05	>0.05		<0.05	<0.05	<0.05	<0.05
				>0.05	>0.05				
C v D	<0.05	>0.05	<0.05	>0.05		<0.05	<0.05	<0.05	

Levels of parameters between genders in HIV infected subjects before and after therapy using 1.5 ng/mL Troponin diagnostic cut-off for myocardial infarction.

Pairwise comparisons showed that the serum level of LDL in male symptomatic HIV infected subjects before antiretroviral therapy was significantly higher compared to female symptomatic HIV infected subjects before antiretroviral therapy ($p<0.05$) (see figure 4.31).

Similarly, the serum activity of AST in male symptomatic HIV infected subjects before antiretroviral therapy was significantly lower compared to female symptomatic HIV infected subjects before antiretroviral therapy ($p<0.05$) (see figure 4.32).

But serum HDL level was the same between sex compared with symptomatic HIV infected subjects before antiretroviral therapy and after commencement of therapy followed up for 12 months ($p>0.05$) using 1.5 ng/mL Troponin diagnostic cut-off for myocardial infarction (see figure 4.33).

Also, the serum activity of Total CK in male symptomatic HIV infected subjects before antiretroviral therapy was significantly higher compared to female symptomatic HIV infected subjects before antiretroviral therapy ($p<0.05$) (see figure 4.34).

Again, Pairwise comparisons showed that the serum activity of LDH in male symptomatic HIV infected subjects before antiretroviral therapy was significantly lower compared to female symptomatic HIV infected subjects before antiretroviral therapy ($p<0.05$) (see figure 4.35).

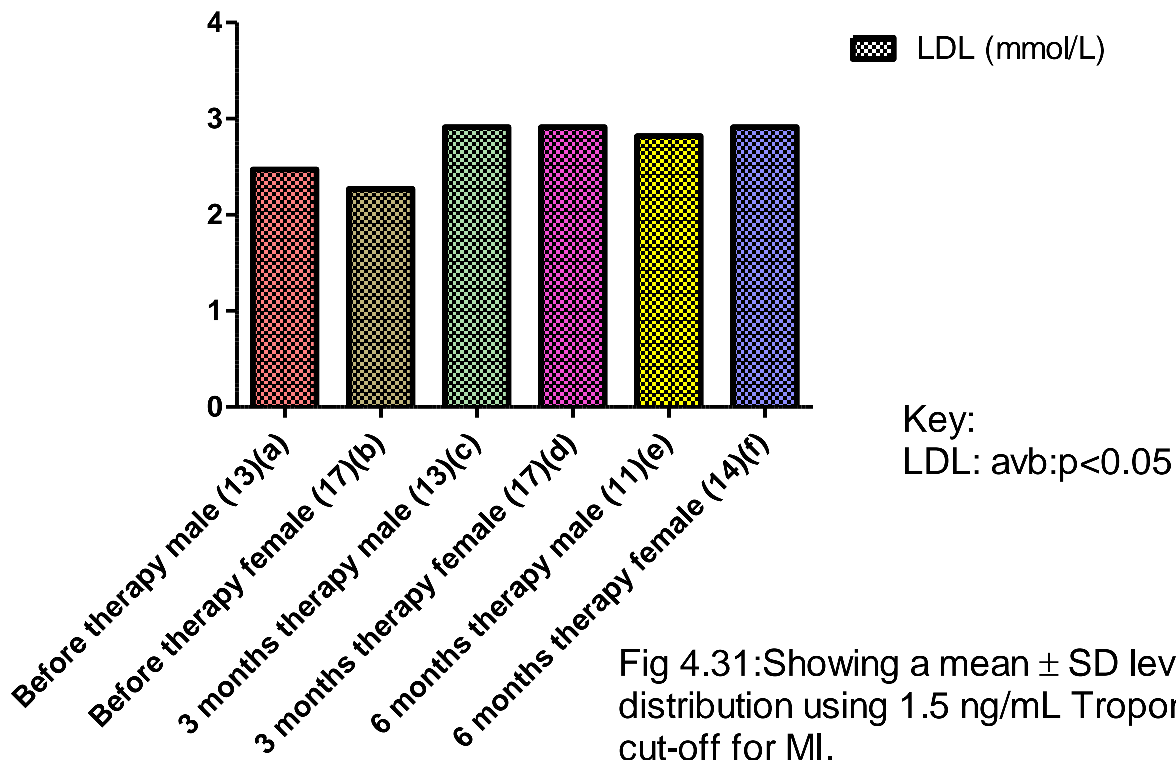


Fig 4.31:Showing a mean ± SD level of LDL in sex distribution using 1.5 ng/mL Troponin diagnostic cut-off for MI.

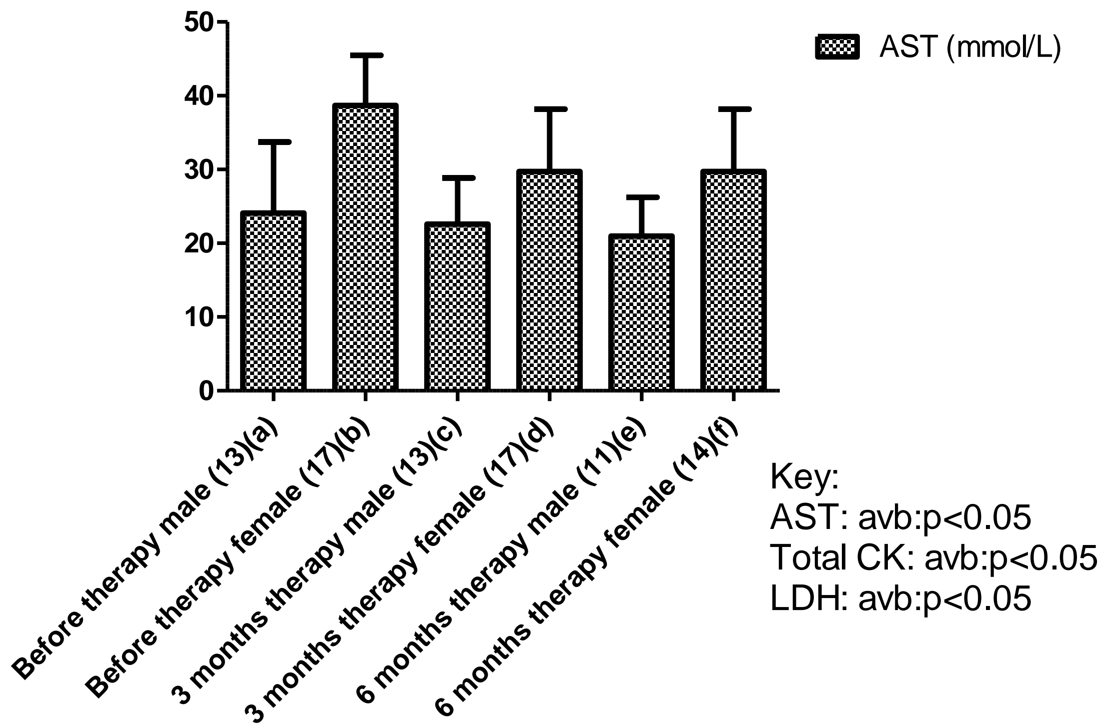


Fig 4.32:showinga mean ± SD level of AST in sex distribution using 1.5 ng/mL Troponin diagnostic cut-off for MI.

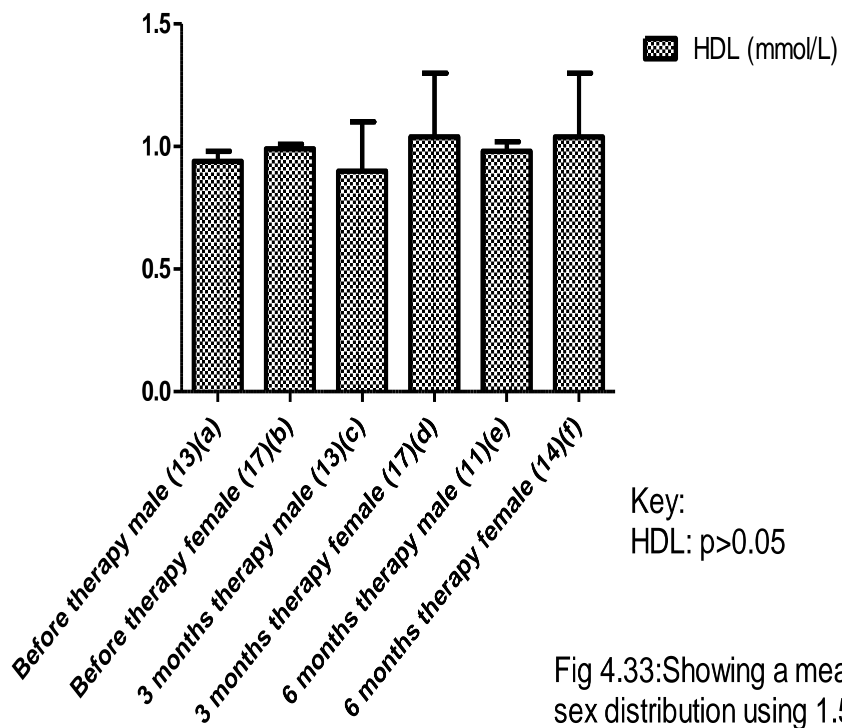


Fig 4.33:Showing a mean ± SD level of HDL in sex distribution using 1.5 ng/mL Troponin diagnostic cut-off for MI.

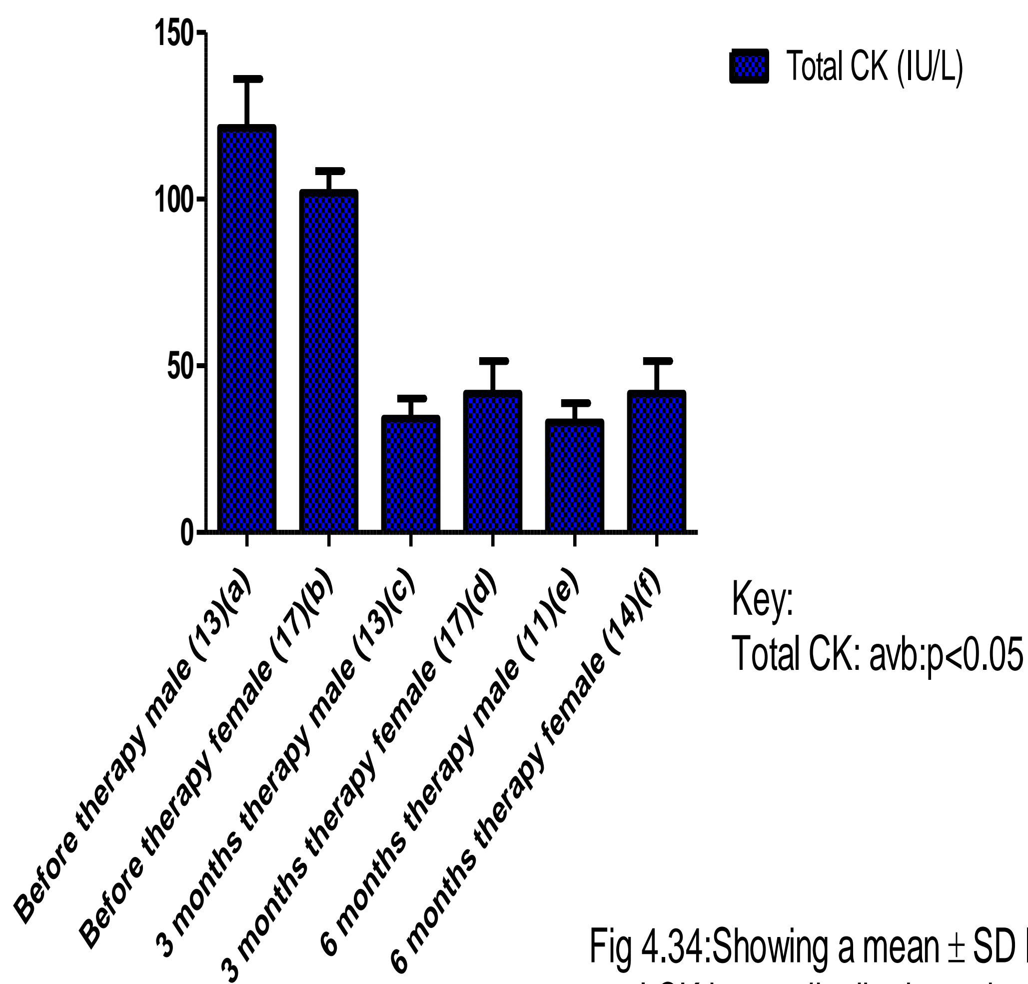


Fig 4.34:Showing a mean ± SD level of total-CK in sex distribution using 1.5 ng/mL Troponin diagnostic cut-off for MI.

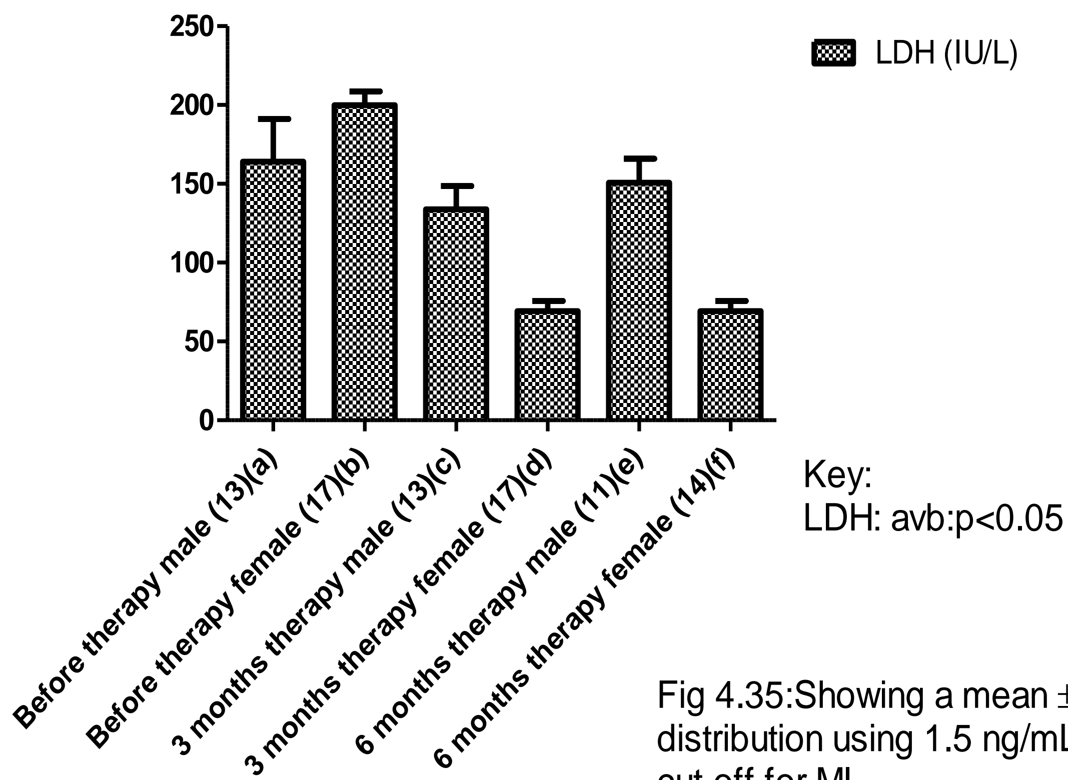


Fig 4.35:Showing a mean ± SD level of LDH in sex distribution using 1.5 ng/mL Troponin diagnostic cut-off for MI.

4.7: Levels of association between parameters studied in symptomatic HIV subjects before therapy using 1.5 ng/mL Troponin diagnostic cut-off for myocardial infarction.

In this study, based on 1.5 mg/ml Troponin diagnostic cut-off for myocardial infarction, in HIV infected subjects before therapy, there was significant positive correlation between Apo A_1 versus TG ($r = 0.012$; $P < 0.05$) , Apo A_2 versus Myoglobin ($r = 0.034$; $P< 0.05$), Apo B versus total Cholesterol ($r = 0 .019$; $P< 0.05$), Apo E versus total Cholesterol ($r = 0 .041$; $P< 0.05$), Apo E versus HDL ($r = 0 .000$; $P< 0.05$), Troponin versus LDL ($r = 0 .036$; $P< 0.05$) and Myoglobin versus LDL ($r = 0 .001$;$P<0.05$). But, there was significant negative correlation between serum Apo A_1 versus LDL ($r = -0.024$; $P< 0.05$), Apo A_2 versus HDL ($r= -0.015$; $P< 0.05$), Apo B versus LDL ($r = -0.029$; $P< 0.05$), Apo C_3 versus Troponin ($r = -0.032$, $P< 0.05$) and Apo E versus LDL ($r = -0.007$; $P< 0.05$) (see table 4.7).

4.8 Levels of association between parameters studied in symptomatic HIV subjects on three months therapy using 1.5 ng/mL Troponin diagnostic cut-off for myocardial infarction.

In this study, based on 1.5 mg/ml Troponin diagnostic cut-off for myocardial infarction, in HIV infected subjects on 3 months therapy, there was significant positive correlation between serum Apo B versus DBP ($r = 0.047$; $P< 0.05$), Apo C_2 versus DBP ($r = 0.000$; $P < 0.05$). However, there was significant negative correlation between serum Apo A_2 versus HDL ($r = -0.039$; $P < 0,05$), Apo C_2 versus LDL ($r = -0.030$; $P< 0.05$), Apo C_2 versus HDL ($r = -0.039$; $P< 0.05$) and Apo C_2 versus TG ($r= -0.033$; $P< 0.05$) (see table 4.8).

4.9: Levels of association between parameters studied in symptomatic HIV subjects on six months therapy using 1.5 ng/mL Troponin diagnostic cut-off for myocardial infarction.

In this study, based on 1.5 mg/ml Troponin diagnostic cut-off for myocardial infarction, in HIV infected subjects on 6 months therapy, there was significant positive correlation between serum Apo A_1 versus HDL ($r =0 .011$; $P< 0.05$), Apo E versus HDL ($r = 0.030$; $p< 0.05$) and Troponin I versus TG ($r = 0.046$; $P < 0.05$). But there was significant negative correlation

between Apo E versus LDL (r = -0.021; P <0.05) and Apo E versus Troponin (r = -0.027; P< 0.05) (table 4.9).

4.10 Levels of association between parameters studied in symptomatic HIV subjects on nine months therapy using 1.5 ng/mL Troponin diagnostic cut-off for myocardial infarction.

In this study, based on 1.5 mg/ml Troponin diagnostic cut-off for myocardial infarction, in HIV infected subjects on 9 months therapy, there was significant negative correlation between serum Apo A_1 versus TG (r = -0.041; P <0.05), Apo C_2 versus HDL (r =- 0.031; P <0.05) and Troponin versus total Cholesterol (r = -0.013; P <0.05) (see table 4.10).

Table 4.7: Levels of association between parameters studied in HIV subjects before therapy using 1.5 ng/mL Troponin diagnostic cut-off for myocardial infarction.

Parameters	Subjects	Correlation coefficient perason r	f-value	p-value
Apo A_1 V LDL	(n=20)	-0.024	0.921	<0.05
Apo A_1 V TG	(n=20)	0.012	0.961	<0.05
Apo A_2 V HDL	(n=20)	-0.015	0.949	<0.05
Apo A_2 V Myoglobin	(n=20)	0.034	0.888	<0.05
Apo B V Cholesterol	(n=20)	0.019	0.935	<0.05
Apo B V LDL	(n=20)	-0.029	0.904	<0.05
Apo C_3 V Troponin	(n=20)	-0.032	0.894	<0.05
Apo E V Cholesterol	(n=20)	0.041	0.863	<0.05
Apo E V LDL	(n=20)	-0.007	0.976	<0.05
Apo E V HDL	(n=20)	0.000	0.998	<0.05
Troponin V LDL	(n=20)	0.036	0.880	<0.05
Myoglobin V LDL	(n=20)	0.001	0.998	<0.05

Table 4.8: Levels of association between parameters studied in HIV subjects on three months therapy using 1.5 ng/mL Troponin diagnostic cut-off for myocardial infarction.

Parameters	Subjects	Correlation coefficient perason r	f-value	p-value
Apo A_2 V HDL	(n=15)	-0.039	0.891	<0.05
Apo C_2 V LDL	(n=15)	-0.030	0.915	<0.05
Apo C_2 V HDL	(n=15)	-0.039	0.891	<0.05
Apo C_2 V TG	(n=15)	-0.033	0.908	<0.05

Table 4.9: Levels of association between parameters studied in HIV subjects on six months therapy using 1.5 ng/mL Troponin diagnostic cut-off for myocardial infarction.

Parameters	Subjects	Correlation coefficient perason r	f-value	p-value
Apo A_1V HDL	(n=14)	0.011	0.970	<0.05
Apo E V LDL	(n=14)	-0.021	0.943	<0.05
Apo E V HDL	(n=14)	0.030	0.918	<0.05
Apo E V Troponin	(n=14)	-0.027	0.928	<0.05
Troponin V TG	(n=14)	0.046	0.876	<0.05

Table 4.10: Levels of association between parameters studied in HIV subjects on nine months therapy using 1.5 ng/mL Troponin diagnostic cut-off for myocardial infarction.

Parameters	Subjects	Correlation coefficient perason r	f-value	p-value
Apo A_1 V TG	(n=4)	-0.040	0.959	<0.05
Apo C_2 V HDL	(n=4)	-0.030	0.969	<0.05
Troponin V Cholesterol	(n=4)	-0.013	0.987	<0.05

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 HIV, antiretroviral therapy and malaria:

The present study showed that the serum concentrations of Apo A_1, Apo A_2, Apo B, Apo C_2, Apo C_3 and Apo E were significantly different in HIV positive individuals. Apo A_1 and Apo C_3 serum concentration decreased significantly in symptomatic HIV subjects not on ART.

This decreased reduction in serum concentration of Apo A_1 may affect the structural composition of HDL, since it is the major apolipoprotein in HDL (Srivastava and Srivastava, 2000). Also, the activity of lecithin cholesterol acyl transferase (LCAT) may be affected. LCAT functions in the removal of excess cholesterol from tissue which is incorporated into HDL and transported to the liver for excretion. Again, for LCAT to function properly, it needs Apo A_1 as a cofactor (Philip *et al*, 1998). Low serum Apo A_1 has been reported to increase coronary heart disease (CHD) as well as in the diagnosis of hyperlipoproteinaemia (Sakurabayashi *et al*, 2001).

The lower level of serum Apo C_3 observed may hinder lipolysis of TG-rich lipoproteins (Jong and Havekes, 2000), thereby leading to higher serum concentrations of TG-rich lipoproteins.

The present study showed significantly higher srum levels of Apo $A_{2,}$ Apo B & Apo E in symptomatic HIV individuals not on ART.

The higher level of Apo A_2 might cause the deposition of abnormal proportion of Apo A_2 in HDL, thereby affecting the normal functioning of HDL in removing “bad cholesterol” from the system. This finding may imply that HIV infection plays a role in affecting the normal structural composition of HDL which may lead to the dyslipidemia in HIV individuals.

Also, the higher serum level of Apo B observed may indicate a pathophysiological role in cardiovascular function. This is in agreement with other reports that excess serum Apo B had been found to be a better predictor of cardiovascular disease (Walldius *et al*, 2001).

Hyperbetalipoproteinaemia has been associated with increased LDL Apo B-100 form, with normal or moderately increased serum concentration of LDL- cholesterol (Sakurabayashi *et al*, 2001).

In this study, the increased level of Apo E observed may explain the wasting disease found in HIV individuals and might also be due to the increased activity of TG-rich lipoprotein catabolism in their body which may be as a result of the effect of the HIV infection on them. Apo E is an essential apolipoprotein for the normal catabolism of TG-rich lipoprotein constituents. Early studies suggested that an increased Apo E level was found to increase hepatic synthesis of VLDL and decrease clearance of triglycerides; these are contributing factors that caused lipid abnormalities (Grunfeld *et al*, 1997). It is part of the composition of chylomicrons, VLDL and HDL (Sakurabayashi *et al*, 2001).

Apo E has been reported to influence psoriasis risk- an autoimmune disease having chronic recurring reddish patches covered with white scales, which is one of the risk factors for hyperlipidemia (Harthi *et al*, 2014). Psoriasis causes abnormal lipid metabolism with a tendency toward an increased shedding off by the skin, total Chol, TG and reduction in HDL levels (Pietrzak and Lecewics *et al*, 2002; Pietrzak *et al*, 2009).

A reduced serum Apo A_2 and increased levels of Apo A_1, Apo B, Apo C_2 (Apo C_2 is a major activator of lipoprotein lipase (LL) and hence stimulates TG hydrolysis (Brawer, 1997)) and Apo E were seen in symptomatic HIV subjects on ART. There are reports that antiretroviral therapy also has the potentials on their own to cause syndrome of fat redistribution, hyperlipidemia and insulin resistance (Lichenstein *et al,* 2005).

The present study observed significantly lower level of lipid profile in symptomatic HIV participants not on ART. The results conformed to those by Grunfeld *et al* (1992) and Ezeugwunne *et al* (2014). Lower level of lipid profile was also observed in Asymptomatic HIV subjects. These reductions could be due to impaired rate of lipid production and enhanced lipid catabolic rate associated with HIV infection (Madhavi *et al*, 2009). HIV infection had been reported to have affected the adipocyte function fats cells where fats are

stored, thereby causing fat redistribution (fat accumulation/ lipodystrophy) (Ahima and Flier, 2000). HIV infection has been reported to cause loss of fat stores under the skin termed lipoatrophy (Hansen *et al*, 2006).

The finding in this study showed increased level of serum total cholesterol, serum LDL and serum Triglyceride and lower level of HDL in symptomatic HIV participants on ART. High serum total cholesterol and Triglyceride levels (dyslipidemia) have been reported in HIV infected individuals on ART (Boyle, 2002; Ogundahunsi *et al*, 2008). These levels are often higher in people with lipodystrophy. Also, higher serum levels of total cholesterol, LDL, Triglyceride and lower level of HDL have been reported to result in cardiovascular risk in individuals (Ahaneku *et al*, 2001; Kabiri *et al*, 2010). Other Authors reported that lipohypertrophy or lipodystrophy was more common but fat loss or lipotrophy was more common in HIV infected subjects on thymidine analogues (Lichenstein *et al*, 2005).

Some researchers have reported that the presence of dyslipidemia in HIV infection might be due to the effects of viral infection, acute-phase reactant and circulating cytokines (Christeff *et al*, 2002). In a study, myocardial infarction, ischemia and coronary artery diseases were observed in HIV positive individuals on ART (D' Armino *et al,* 2004).

In this study, the serum levels of Myoglobin, Troponin I, CK-T, CK-MB, LDH and AST were significantly higher in HIV positive individuals and more marked in symptomatic HIV individuals not on ART. The serum levels of Myoglobin, Tropinin 1 and serum activities of total CK, CK-MB and LDH were significantly higher in HIV positive individuals (Ezeugwunne *et al,* 2014). The above outcome may be explained by the effect of HIV virus on the myocardium leading to low immunity (Malnich and Goland, 1998), inflammatory response of host myocardium to the virus (Lewis, 2000), heart problem such as pericarditis (Sudano *et al*, 2006) and endocarditis (Miro *et al*, 2003).

The significantly higher levels of Troponin I, CK-T, CK-MB and AST observed in symptomatic HIV individuals on ART when compared to control may be due to antiretroviral

therapy from the triple therapy from zidovudine, lamivudine and nevirapine. There are reports that ART predisposes one to heart disease (Freichs *et al*, 2002).

In this study, the CD4 T cell count was significantly depleted in symptomatic HIV individuals not on ART, however, improved value was observed in those o antiretroviral therapy, thus suggesting possible immune recovery from the therapy. Ifeanyichukwu *et al*, (2011) was able to link rate of CD4+ T cell depletion with HIV disease progression while Mark *et al*, (2005) artributed the cause of CD4 depletion in HIV seropositives to cell death by HIV infection.

There were increased serum levels of Apo C_3 and CK-MB but decreased levels of Apo E, HDL and CD4 counts in symptomatic HIV on ART with malaria parasitaemia compared to those without malaria parasitaemia. The elevated levels of Apo C_3 and CK-MB and lower level of CD4 may be due to adverse effect of malaria infection on the organs (Poddutoor *et al,* 2010). Onyenekwe *et al*, (2007) reported a tripled prevalence of malaria infection in symptomatic HIV infected subjects. Ezeugwunne *et al*, (2012) observed that HIV and malaria co-infection were capable of lowering the value of CD4 counts in HIV individuals.

There were increased serum levels of Apo E, TG and Myoglobin but decreased levels of Apo C_2, and HDL in symptomatic HIV not on ART with malaria parasitaemia compared to those without malaria parasitaemia. Poddutor *et al* (2010) reported an adverse effect of malaria infection on body organs.

The study also showed increased serum levels of Apo E and Troponin I in Asymptomatic HIV infected subjects with malaria infection compared to those without malaria parasitaemia. Again, *Plasmodium falciparum* has been shown to stimulate HIV replication through the production of cytokines by activated lymphocytes (Frobel *et al*, 2004). This may suggests that malaria may speed up the progression of HIV disease (Kublin *et al*, 2005).

Results obtained from symptomatic HIV subjects not on ART that were later monitored when they commenced therapy for a period of 12 months showed that the serum level of Apo A_1 was significantly higher in symptomatic HIV subjects on 3, 6, 9 and 12 months therapy

when compared with value before therapy. These increased levels of serum Apo A_1 observed with lengthened therapy might suggest immune recovery, impaired by HIV infection and cardioprotective role (Sakurabayashi *et al,* 2001). The implication might lead to normal restructuring of HDL by Apo A_1 (Srivastava and Srivastava, 2000), there by stimulating normal activity of LCAT, as Apo A_1 serves as a cofactor for LCAT in removing excess Cholesterol from tissue (Philip *et al,* 1998). Apo A_1 is often used as a biomarker for cardiovascular diseases (McQueen *et al*, 2008). The value of serum Apo A_1 was significantly higher in symptomatic HIV female subjects than in male before therapy and at 3 months therapy.

Again, the study showed that the serum level of Apo A_2 was significantly lower in symptomatic HIV subjects on 3, 6, 9 and 12 months therapy when compared with value before therapy. Normal serum Apo A_2 will increase the rate of hepatic and lipoprotein lipase activity, the effect which tends to increase plasma TG hydrolysis and thus reduce plasma TG (Kalopissis and Chambaz, 2000). There was no sex effect on the value of serum Apo A_2 in these subjects.

Also, the study showed that the serum level of Apo B was significantly higher in symptomatic HIV subjects on 3, 6 and 9 months therapy when compared with value before therapy. It is worthy to note that there was significant reduction in serum level of Apo B from 3 months therapy to 12 months therapy. This may indicate cardio-protectiveness of Apo B. There was no sex effect on the value of serum Apo B in these subjects. Excess Apo B in individual has been found to be a better predictor of cardiovascular disease (Walldius *et al,* 2001).

Based on the study, there was a sharp reduction of serum level of Apo C_2 from before therapy to 3 months in symptomatic HIV infected subjects. The value significantly rises from 3 months to 9 months therapy. There was no sex effect on the value of serum Apo C_2 in these subjects. Elevated serum Apo C_2 level has been linked with hypertriglyceridemia, hypercholesterolemia and hyperchylomicronemia (Jackson *et al,* 1977).

But there was a sharp increase of serum level of Apo C_3 from before therapy to 3 months in symptomatic HIV infected subjects. The value significantly reduces from 3 months to 9 months therapy. There was no sex effect on the value of serum Apo C_3 in these subjects. Elevated level of serum Apo C_3 may be linked with coronary heart diseases (Singh *et al*, 2008).

In this study, the serum Apo E level was significantly reduced in symptomatic HIV infected subjects as the duration of therapy increased when compared to the value before therapy. There was no sex effect on the value of serum Apo E in these subjects. Apo E protein is found in chylomicrons and intermediate lipoproteins (IDLs) that is essential in the catabolism of triglycerides-rich lipoprotein constituents and it is essential in the transport of cholesterol to neurons via Apo E receptors (Singh *et al*, 2002). There is evidence that Apo E protects against atherogenesis, hence the reduced value of Apo E observed as the duration of therapy increased may indicate a cardio-protective role on the heart (Larkin *et al*, 2000). Also, there have been reports on the association between Apo E and neurodegenerative conditions such as multiple sclerosis and Alzheimer's disease (Fazekes *et al*, 2011).

Also, the lipid profile was significantly higher in symptomatic HIV infected subjects as length of therapy deepened when compared to value before therapy. Dyslipidemia –an elevated serum total cholesterol and triglycerides levels have been reported in HIV infected subjects on antiretroviral therapy (Boyles, 2002; Ogundahunsi *et al*, 2008; Ezeugwunne *et al*, 2014). Elevated levels of total Cholesterol, triglycerides and LDL observed in these subjects as therapy deepened may predispose them to cardiovascular diseases. Elevated levels of total Cholesterol, triglycerides and LDL have been reported to cause cardiovascular diseases (Ahaneku *et al*, 2001; Kabiri *et al*, 2010). Antiretroviral therapy has been linked with hyperlipidemia (Parazaket *et al,* 2002). Some reported mechanism of dyslipidemia include significant decreases in hepatic and lipoprotein lipase activities (Pumell *et al,* 2000) or low fat clearance owing to impaired lipoprotein lipase-mediated clearance of triglycende-rich lipoprotein (Sekhar *et al*, 2005; Shahmanesh *et al*, 2005)

Furthermore, there was no sex effect on value of total cholesterol in this study. The value of serum LDL was significantly higher in male than female symptomatic HIV infected subjects before therapy and at 6, 9 and 12 months therapy. An increased LDL level is a strong predictor for cardiovascular diseases (Riddler *et al*, 2003). This may confirm that that male sex is more prone to cardiovascular diseases (Kabiri *et al*, 2010). Also, the value of serum HDL was significantly higher in female than in male symptomatic HIV infected subjects on 6, 9 and 12 months therapy. Although, the elevated level of HDL observed in these subjects as the duration of therapy increased may indicate cardio-protectiveness on the host. The value of TGs was significantly higher in male than in female symptomatic HIV infected subjects on 6 and 9 months therapy.

In this study, the activity of serum CK-MB and levels of Myoglobin and Troponin I were sharply increased from before therapy to 3 months therapy in symptomatic HIV infected subjects. The values were significantly reduced from 3 months to as length of therapy lengthened. The sharp reduction of values observed at 3 months may be as a result of the therapeutic effect of the drug on the heart and with more reduction in values of these parameters as length of therapy elongated may indicate cardio-protectiveness on the host. The activity of serum total CK was significantly reduced as length of therapy deepened. This may still connote recovery and cardio-protectiveness of the drug on the heart. Myoglobin is useful for early diagnosis of acute myocardial infarction. It rises within 2-4 hours after the early diagnoses of acute myocardial infarction. It peaks at 9-12 hours and returns to baseline within 24-36 hours (Kegen, 1978; Silva *et al*, 1991).

It has been reported that acute myocardial infarction (AMI) might occur at 1.5ng/ml Troponin level (NCLLS, 1997). The level of Troponin I in this symptomatic HIV infected subjects at 3 months therapy was higher than the actual value for Troponin diagnostic cut-off value for AMI. Conditions such as unstable angina, myocarditis, and congestive heart failure have been documented to cause myocardial cell damage with potentially increased cardiac Troponin l levels above the expected value (Adams *et al*, 1993). The most preferred biomarker for

myocardial damage is cardiac Troponin (I or T) (Eisenman, 2006). The cardiac Troponins I or T are released within 4 - 6 hours of an attack of MI and remain elevated for up to 2 weeks with peak concentrations reached in approximately 12 to 24 hours after infarction and are now preferred markers for assessing myocardial damage (Ebell *et al*, 2000; Eisenman , 2006). CK –MB is the isoform of CK found in the heart and it is sensitive to MI just as where the levels of troponin I and myoglobin. Eisenman, (2006) reported that serum Troponin, Myoglobin and CK-MB were elevated in acute ischemia such as AMI.

Again, the CD4 count was sharply reduced from before therapy to 3 months therapy in symptomatic HIV infected subjects and significantly increased from 3 months to as the duration of therapy increased. The sharp reduction may be as a result of the effect of both the disease burden and drug toxicity on the immune system. The increased values observed with length of therapy may indicate immune recovery on the host.

There was no sex effect on the value of serum total CK and CK-MB in symptomatic HIV infected subjects before and after therapy. However, the value of Myoglobin was significantly higher in male than female symptomatic HIV infected subjects on 3 and 6 months therapy. The value of Troponin 1 was significantly higher in male than female symptomatic HIV infected subjects on 3 months therapy but lower in male than female symptomatic HIV infected subjects on 6 months therapy. Similarly, the value of CD4 count was significantly higher in female than male symptomatic HIV infected subjects before therapy but lower in female than male symptomatic HIV infected subjects on 12 months therapy. Antiretroviral therapy has been found to reduce viremia (Dubul *et al,* 2002). This implies that an immune recovery by the antiretroviral therapy.

The main obstacle to ART eliminating HIV infection is that HIV is able to integrate itself into the DNA of host cells and rest in a latent state known as viral reservoirs, which served as central memory and transitional memory CD4 cells while antiretrovirals only attack actively replicating HIV (Chomont *et al*, 2009). Meanwhile, there is work being done to try and

activate reservoir cells into replication so that the virus is forced out of latency and can be attacked by antiretrovirals and the host immune system (Archin *et al*, 2010).

Results obtained from symptomatic HIV subjects not on ART that were later monitored when they commenced therapy for a period of 12 months were reclassified using 1.5ng/ml Troponin diagnostic cut-off for myocardial infarction (NCCLS, 1997), the serum levels of Apo A_1, Apo B and Apo C_3, total cholesterol, HDL-C, Myoglobin, Troponin 1 and activity of CK-MB were sharply increased at 3 months therapy compared to values before therapy. This may indicate possible myocardiac infarction at this stage of therapy, implying that early therapy may predisposes individual to MI due to the toxic effect of the drugs.

Based on 1.5ng/ml Troponin diagnostic cut-off for myocardial infarction, the serum levels of Apo $A_{2,}$ Apo C_2, Apo E, total CK and LDH were sharply reduced at 3 months therapy compared to values before therapy. These reductions observed may indicate possible cardio-protective effect of the drug on the release ofthese biomarkers in the heart.

Results from 1.5ng/ml Troponin diagnostic cut-off for myocardial infarction, showed that the serum levels of Apo $A_{1,}$ Apo $A_{2,}$ Apo B, Apo C_2, Apo E and lipid profile were significantly increased as length of therapy elongated.

The serum levels of Apo $A_{1,}$ Apo $A_{2,}$ Apo B, Apo C_2, Apo C_3, Apo E, Myoglobin, total CK, CK-MB and CD4 were improved as length of therapy elongated. This may connote immune recovery and therapeutic role of the drugs on the heart. But the lipid profile and Troponin1 were significantly increased as length of therapy elongated. The implication may be that they are more sensitive to cardiovascular event and best biomarkers for myocardial infarction.

The value of LDL was significantly higher in male than female symptomatic HIV infected subjects before therapy only. The activity of total CK was significantly higher in male than female symptomatic HIV infected subjects before therapy but lower in male than female symptomatic HIV infected subjects on 3 and 6 months therapy. The activity of total LDH was significantly higher in female than male symptomatic HIV infected subjects before therapy

but lower in female than male symptomatic HIV infected subjects on 3 and 6 months therapy. There was sex effect on HDL in these subjects.

In this study, based on 1.5 ng/ml Troponin diagnostic cut-off for MI, there was significant positive correlation between serum Apo A_1 versus TG, Apo A_2 versus Myoglobin, Apo B versus total Cholesterol, Apo E versus total Cholesterol, Apo E verus HDL, Troponin versus LDL and Myoglobin versus LDL in symptomatic HIV infected subjects before therapy. The positive correlation denotes that as one parameter is increasing the other is also increasing. Also, the was significant negative correlation between serum Apo A_1 versus LDL, Apo A_2 versus HDL, Apo B versus LDL, Apo C_3 versus Troponin and Apo E versus LDL in symptomatic HIV infected subjects before therapy. The negative correlation denotes that as one parameter is decreasing the other is also increasing. These correlations could serve as a predictive or prognostic index for evaluating MI in HIV infected subjects. The information extricated from these correlations could help in determining when to start administering lipid lowering drugs as well as cardiovascular drugs to prevent coronary heart disease.

Using the 1.5 ng/ml Troponin diagnostic cut-off for MI, there was significant negative correlation between serum Apo A_2 versus HDL, Apo C_2 versus LDL, Apo C_2 versus HDL and Apo C_2 versus TG in symptomatic HIV infected subjects on 3 months therapy. These parameters could help in evaluating MI in HIV infected subjects on antiretroviral therapy.

There was significant positive correlation between serum Apo A_1 versus HDL, Apo E versus HDL and Troponin versus TG in symptomatic HIV infected subjects on 6 months therapy. Also, there was significant negative correlation between serum Apo E versus LDL and Apo E versus Troponin in symptomatic HIV infected subjects on 6 months therapy. This is strongly suggestive that these parameters could be used in determining the presence of MI in symptomatic HIV infected subjects even when undergoing therapy before and at 6 months.

5.2: Conclusion:

From the results of this study,

The reduction of serum apolipoproteins (Apo A_2, Apo B, Apo C_2,Apo E) and other cardiac markers (CK-T, CK-MB, myoglobin, LDH) in HIV subjects on ART suggest improved cardiac function. However, malaria endemicity poses a threat to the improved cardiac function as observed with increased serum levels of Apo C_3, Apo E, CK-MB, myoglobin, and triglyceride in HIV malaria co-infection with or without ART. This calls for concern in the management of patients with HIV malaria co-infection.

5.3: Recommendation:

It is recommended that HIV infected individuals with two or more cardiac risk factors should have their cardiac risk score calculated by Framing equation, used to provide Guidance for management and treatment of patients with cardiovascular disease (Aberg, 2009). This is to enhance management of HIV infected subjects in our environment.

Lifestyle modifications such as smoking, smoking cessation, diet and exercise should be prescribed. Lipid lowering therapy should be initiated or a switch in antiretroviral therapy should be made from current PIs and NNRTIs to the newly approved classes of integrase inhibitors and entry inhibitors as a means for improving lipid parameters and reducing cardiovascular risk.

Since some markers were observed to be cardioprotective and cardiac destructive, it is then pertinent for health care-givers to support Symptomatic HIV infected subjects with drugs that would take care of cardiovascular risk factors.

Finally, the community health implication of HIV, antiretroviral therapy and malaria infection cannot be overemphasized, especially in Sub-saharan Africa, hence, more researches are needed to be done on the interactions between HIV infection, antiretroviral therapy, Malarial infection and cardiovascular risk factors and attendant diseases/ disorders.

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APPENDIX

NNAMDI AZIKIWE UNIVERSITY TEACHING HOSPITAL

P.M.B. 5025, NNEWI, ANAMBRA STATE, NIGERIA

Prof. S. N. Nnatu
MB, BCH, FWACS, FICS, FMCOG, FRCOG London
Chairman
Board of Management

B.O. Chukwuma
B. Sc., MA, MHA, AHA
Director of Administration/
Secretary to the Board

Prof. R. O. Ofiaeli
MBBS (IB), FMCS, FICS, FWACS,
Chief Medical Director/
Chief Executive

Dr. A. O. Igwegbe
MBBS, FWACS, FICS, FISS
Chairman
Medical Advisory Committee

E-mail: nauthcmd@yahoo.co.uk
nauthnnewi@hotmail.com
Telegram: TEACHOS NNEWI

Our Ref: NAUTH/CS/66/VOL.6/43

Your Ref:

Date: 14th March, 2012

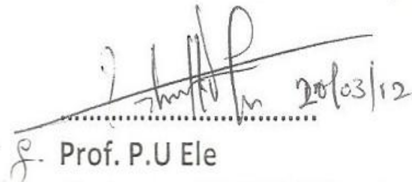
Ezeugwunne Ifeoma Priscilla
Department of Chemical Pathology,
College of Health Sciences,
Nnamdi Azikiwe University,
Nnewi Campus.

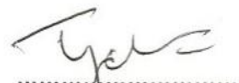
ETHICAL COMMITTEE APPROVAL

RE: EVALUATION OF APOLIPOPROTEIN PROFILES AND OTHER MAKERS OF CARDIAC FUNCTION IN HIV/AIDS PARTICIPANTS ON ART

I write to inform you that after due consideration of your research proposal, approval is hereby conveyed for you to commence the study.

The principal investigator is required to send a progress report to the Ethics Committee at the expiration of three (3) months after ethical clearance to enable the Committee carry out its oversight function.





Prof. P.U Ele
Chairman, NAUTH Ethics Committee

J.U. Ugochukwu (Mrs)
Sec., NAUTH Ethics Committee

DEPARTMENT OF CHEMICAL PATHOLOGY

COLLEGE OF HEALTH SCIENCES

NNAMDI AZIKIWE UNIVERSITY
P.M.B. 5001 NNEWI ANAMBRA STATE NIGERIA

Office of the Head of Department

Tel: 046-463663 Fax:046-460124 E-maill: chsnne@infoweb.abs.net

Our Ref: ______ Your Ref: ______ Date: ______

Dept. of Chem. Path
Faculty of Medicine
College of Health Sciences,
NAU, Nnewi
8/9/14.

The Technical Head,
Dept. of Chem. Path,
NAUTH, Nnewi.

REQUEST FOR NNAEDOZIE OKWUDIRI TO ASSIT EZEUGWUNNE IFEOMA P TO USE ELISA MACHINE

Could you please allow Nnaedozie Okwudiri to assist Ezeugwunne Ifeoma P. in the use of our ELISA Machine for her research analysis.

Thanks

Prof. C. E. Dioka

SITY TEACHING HOSPITAL

IAMBRA STATE, NIGERIA

MB, BCH, FWACS, FICS, FMCOG, FRCOG London
Chairman
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Medical Advisory Committee

E-mail: nauthcmd@yahoo.co.uk
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Telegram: TEACHOS NNEWI

Ref: NAUTH/CS/66/01/012

Ref: ______________________

Date: 2nd February, 2012

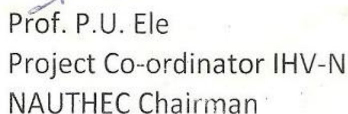
Mrs I.P Ezeugwunne,
Department of Human Biochemistry,
Faculty of Basic Medical Sciences,
Nnamdi Azikiwe University,
Nnewi Campus.

LETTER OF PERMISSION

You are hereby authorized to avail yourself of resources in the HIV Unit for the purposes only of your PhD Programme.

It is understood that this permission is within the context of strict compliance to the NAUTH Ethics Committees requirements as communicated to you. Any breach will attract automatic revocation of this permission.

You will be required to present this letter to the respective focal persons for clearance in their units before actual commencement of your work.



Prof. P.U. Ele
Project Co-ordinator IHV-N
NAUTHEC Chairman

INFORMED CONSENT FORM

I am Ezeugwunne Ifeoma P (Mrs), a Chemical pathologist at the Nnamdi Azikiwe University, Nnewi Campus. I am carrying out a study on the "Evaluation of apolipoprotein profile and other markers of cardiac function in HIV subjects on antiretroviral therapy, in NAUTH Nnewi, South East Nigeria'.

Purpose of study

The spread of retroviral infection can only be controlled, prevented and managed presently since there is yet no cure. In achieving prevention and good control and management understanding of the way this retrovirus acts and how it affects the human functions is important. I want to try and check how the retrovirus affects the human function in infected patients and use the knowledge obtained from the study to enhance good control and management of patients with retroviral infection in this community, state, country and internationally.

Methods

For us to be able to effectively achieve the purpose of the study, I shall be requesting you to allow me get 10 mls of blood from you. This blood sample will be used only for the parameters that will help me understand how the retrovirus acts on the function of the body. Any blood sample left after the laboratory analysis will be properly and safely discarded. We shall also examine your body for any signs and symptoms that will help me in the study because some signs and symptoms are associated with the retroviral infections and may be used to know the extent of the infection.

Subject participation

If you are diagnosed with retroviral infection, I shall highly be interested that you participate in this study. Participation is voluntary. You may withdraw at anytime you no lnger wish to continue. There is no penalty for withdrawal nor will it affect your care.

Confidentiality of information

I am are assuring you that all the information that you supplied to us and those obtained from the blood analysis for the purpose of this study shall be kept confidential no person can use the information to trace you or know who you are.

Risks/ benefits

No risk is involved if you participate in the study. The benefits that you will have from this study are that your personal results can be revealed to you and if you seek advice you will be properly assisted through the facilities available for assistance. Secondly the findings of this will help in either reviewing or upgrading the prevention, control and management policies.

Consent

Statement of the person given consent: I have read the description of the research, I understand that my participation is voluntary. I know enough about the purpose, method, risk and benefits of research study to judge that I want to take part in it. I understand that I may freely stop being part of this study at any time. I have received a copy of this consent form from the additional information sheet to keep for myself.

Date……….. Signature………… Name…………. Phone no………..

QUESTIONAIRE

The following question requires voluntary and sincere response. The information provided is considered highly confidential.

Section A: Biodata

No:

Age:

Occupation:

Religion:

Marital status:

Types of marital union:

Section B: Mode of infection:

When did you test positive?: A month ago; 6 months ago; 12 months ago; > 18 months ago.

Have you had blood transfusion in the past?. Yes/ No.

Do you have injection from patent drug dealers?. Yes/ No.

When do you have unprotected sex?.

a) 0-3 months b) 4-6 months c) 7-12 months d) 13-18 months e) >18 months.

Do you have more than one sex partner?. Yes/No.

Have you been treated for STD?. Yes/ No.

Which of the following symptoms (s) do you experience? a) fever b) rashes c) weight loss d) weakness d) diarrhea e) cough f) dizziness g) oral thrush.

Are you on antiretroviral therapy?. Yes/ No.

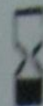
How long have you been on ART?. a) headache b) joint pains c) fever d) weakness.

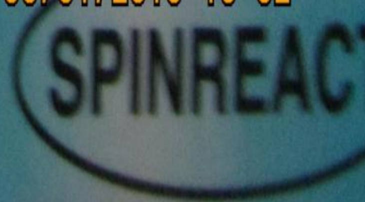
When last were you treated for malaria?. a) A months ago b) 3 months ago c) 6 months ago.

11/08/2012 19:11

11/08/2012 19:51

11/27/2012 17:40





QUALITY CONTROL RESULTS

CK-T U/L

Intral	Inter
67, 17, 21	40, 38, 55
66.5, 17, 20.8	39.6, 38, 55
66.3, 16.7, 20.6	39.3, 37.8, 54.5
66.4, 16.7, 20.6	39.3, 37.5, 54.3
66.4, 16.7, 20.6	39.3, 37.5, 54.5

n= 30, mean= 39.28, SD= 17.7, CV= 45%

CK-MB U/L

2, 2, 3.1	4, 2, 3
1.8, 1.9, 2.9	3.9, 1.7, 2.8
1.7, 1.6, 2.7	3.8, 1.5, 2.7
1.7, 1.8, 2.8	3.8, 1.6, 2.7
1.7, 1.7, 2.7	3.8, 1.5, 2.7

n= 30, mean= 2,45, SD= 0.82, CV= 33.47 %

AST U/L

20, 5.46. 20.7	18, 5.1 , 7
19.8, 5.44, 20.3	17.8, 5, 6.9
19.6, 5.42, 20.3	17.6, 4.8, 6.7
19.6, 5.42, 20.3	17.6, 4.8, 6.7
19.6, 5.42, 20.3	17.6, 4.8, 6.7

n= 30, mean= 12.50, SD=6.98, CV= 55.84 %

LDH U/L

16, 123, 169 116, 169, 171

15.8 ,122.8, 168.7 115.5, 168.5, 170.6

15.6, 122.5, 168.4 115.2, 168.3, 170.4

15.7, 122.6, 168.4 115.2, 168.3, 170.4

15.7, 122.6, 168.4 115.2, 168.3, 170.4

n= 30, mean= 121.34, SD=59.47, CV= 49.01 %

MYOGLOBIN ng/mL

0, 25, 250, 500, 1000 33.5, 19.4, 25

0, 25, 250, 500, 1000 33.4, 19.2, 25

0, 24.8, 249.8, 500, 1000 33.4, 19.2, 25

0, 24.8, 249.8, 500, 1000 33.4, 19.2, 25

0, 24.8, 249.8, 500, 1000 33.4, 19.2, 25

n= 30, mean= 231.54, SD=337.08, CV= 145.58 %

TROPONIN I ng/mL

0, 2, 7.5, 30, 75 0.25, 0.37, 0.39

0, 2, 7.4, 30, 74.9 0.23, 0.35, 0.37

0, 1.9, 7.2, 29.5, 74.7 0.22, 0.33, 0.35

0, 1.9, 7.2, 29.5, 74.8 0.22, 0.34, 0.35

0, 1.9, 7.2, 29.5, 74.7 0.22, 0.34, 0.35

n= 44, mean= 14.34, SD=337.08, CV= 25.05 %

Apo A1 mg/dL

1.08, 1.30, 1.33 1.26, 1.30, 1.24

1.06, 1.28, 1.32 1.25, 1.28, 1.22

1.05, 1.27, 1.30 1.23, 1.26, 1.20

1.05, 1.27, 1.30 1.23, 1.26, 1.20

1.05, 1.27, 1.30 1.23, 1.26, 1.20

n= 30, mean= 1.23, SD=0.08, CV= 6.5 %

Apo A1 mg/dL

29.8, 23, 25 26, 24, 24

29.6, 22.8, 24.8 25.8, 23.7, 23.7

29.4, 22.6, 24.5 25.5, 23.5, 23.5

29.4, 22.6, 24.5 25.5, 23.5, 23.5

29.4, 22.5, 24.5 25.5, 23.5, 23.5

n= 30, mean= 24.97, SD=2.28, CV= 9.13 %

Apo B mg/dL

80, 105, 85 55, 70, 120

79.8, 104.8, 84.8 54.8, 69.8, 121.8

79.5, 104.5, 84.6 54.6, 69.6, 121.5

79.5, 104.5, 84.6 54.6, 69.6, 121.5

79.5, 104.6, 84.6 54.6, 69.6, 121.5

n= 30, mean= 85.79, SD=22.3, CV= 25.99 %

Apo C mg/dL

9.1, 7.4, 5 1, 2, 4

9, 7.3, 4.8 0.9, 1.8, 3.8

8.9, 7.2, 4.6 0.7, 1.6, 3.6

8.9, 7.2, 4.6 0.7, 1.6, 3.6

8.9, 7.2, 4.6 0.8, 1.6, 3.7

n= 30, mean= 4.54, SD=2.29, CV= 64 %

Apo E mg/dL

4.4, 3.3, 3 5.1, 4, 6.2

4.3, 3.2, 2.8 5, 3.9, 6.1

4.1, 3.1, 2.7 4.9, 3.7, 6

4.2, 3.1, 2.8 4.9, 3.7, 6

4.2, 3.1, 2.7 4.9, 3.7, 6

n= 30, mean= 4.17, SD=1.12, CV= 26 %

Total Cholesterol mmol/L

5.18, 4.98, 5.49 5.50, 5.30

5.18, 4.98, 5.49 5.50, 5.30

5.18, 4.98, 5.49 5.50, 5.30

5.17, 4.97, 5.48 5.50, 5.30

5.17, 4.97, 5.48 5.50, 5.30

n= 25, mean= 5.29, SD=0.20, CV= 3.78 %

TG mmol/L

2.26, 1.68, 1.70 1.73, 1.76

2.26, 1.68, 1.70 1.73, 1.76

2.25, 1.68, 1.70 1.73, 1.76

2.25, 1.67, 1.69 1.72, 1.75

2.25, 1.67, 1.69 1.72, 1.75

n= 25, mean= 1.82, SD=0.22, CV= 12.09 %

HDL mmol/L

1.36, 0.89, 0.85	0.75, 0.80
1.36, 0.89, 0.85	0.75, 0.80
1.35, 0.89, 0.85	0.75, 0.80
1.35, 0.88, 0.84	0.75, 0.80
1.35, 0.88, 0.84	0.75, 0.80

n= 25, mean= 0.93, SD=0.22, CV= 23.66 %