KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

KUMASI

COLLEGE OF HEALTH SCIENCE

SCHOOL OF MEDICAL SCIENCE

DEPARTMENT OF MOLECULAR MEDICINE

NEOPTERIN AS A SURROGATE MARKER FOR MONITORING HIV DISEASE PROGRESSION AND PATIENT'S RESPONSE TO ANTIRETROVIRAL THERAPY: A CASE CONTROL STUDY IN KUMASI, GHANA

A THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF PHILOSOPHY DEGREE IN CHEMICAL PATHOLOGY AT THE DEPARTMENT OF MOLECULAR MEDICINE,

SCHOOL OF MEDICAL SCIENCES

BY

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DECLARATION

With the exception of acknowledged statements, I hereby declare that this is my own research work carried out at the Bomso Specialist Hospital and has not been published nor contains materials towards an award of a degree in any university.

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DEDICATION

This thesis is dedicated to my parents, Mr. Joseph Boafo and Mrs. Agartha Boafo and my friend Dan Quansah Yedu

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To God be the Glory, Great things He has done. I owe it all to him for his goodness, kindness and for his endless mercies. All his ways are pleasant and his works are of peace.

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ABSTRACT

Background: Surrogate markers including CD4 T cell, viral load estimation and activated immune markers have been identified as having significance in the pathogenesis and prognosis of the HIV infection. But there is limited data on the utility of serum neopterin estimation in HIV infection.

Aim: This study was aimed at evaluating serum neopterin levels as a marker of predicting the progression of HIV infection and to monitor patient's response to antiretroviral therapy.

Method: In all one hundred and ninety two (192) HIV infected patients constituting one hundred and four highly active antiretroviral therapy patients (104) and eighty eight (88) highly active antiretroviral therapy naïve patients were recruited from the HIV clinic at the Bomso specialist hospital in the Kumasi metropolis. Venous blood samples were taken and assayed for haematological parameters (Haemoglobin, White blood cell, Red blood cell, Mean cell haemoglobin, Mean cell volume, Haematocrit and Mean cell haemoglobin concentration) , Biochemical parameters (Albumin , Aspartate amino transferase, Alanine amino transferase , urea, creatinine, total protein and globulin), Immunological parameters (CD4 count and neopterin). Weight and height were also taken for the determination of body mass index.

Results: Out of the one hundred and four (104) highly active antiretroviral therapy patients, majority (57.7%) were on Tenofivir, Lamivudine and Nevirapine combination whiles only 5.8% were on Zidovudine, Lamivudine and Nevirapine combination. Although the median age of the highly active antiretroviral therapy patients (41yrs) was higher than the highly active antiretroviral therapy naïve patients (40yrs), this was statistically not significant (p=0.203). Haematological analysis also showed a significant increase (p=0.001) in haemoglobin levels in the highly active antiretroviral therapy patients (12.40g/dL) compared to the highly active antiretroviral therapy naïve patients (11.60g/dL). A correlation analysis between CD4 count and haemoglobin levels of the highly active antiretroviral therapy naïve patients showed a significant positive correlation which indicates the usefulness of haemoglobin measurement in the management of HIV infected patients. Result from the biochemical assay showed a significant difference in albumin levels (p=0.0001) in patients placed on highly active antiretroviral therapy (40.05g/L) compared to the highly active antiretroviral therapy naïve patients (34.75g/L). Serum total protein, globulin, Aspartate amino transferase, Alanine amino transferase, urea and creatinine were significantly

lower (p=0.0001) in the highly active antiretroviral therapy patients compared to the naïve highly active antiretroviral therapy patients. A statistically significant negative correlation was observed between the duration of therapy treatment and the transaminases (Aspartate amino transferase and Alanine amino transferase). Although body mass index was statistically not significant (p=0.521) between the highly active antiretroviral therapy patients and the naïve highly active antiretroviral therapy patients, patients on the highly active antiretroviral therapy had a higher body mass index (23.30kg/m^2) compared to the naïve highly active antiretroviral therapy patients (22.55kg/m^2). Neopterin was significantly lower (p=0.0001) in patients placed on the highly active antiretroviral therapy (26.40nmol/L) than the naïve highly active antiretroviral therapy patients (51.75nmol/L). The study also revealed a strong negative correlation between serum neopterin and CD4 count for both the highly active antiretroviral therapy patients (rho-0.99, p=0.0001) and the naïve highly active antiretroviral therapy patients (rho -0.96, p=0.0001). Serum neopterin were found to be increased as the disease progresses in the studied participants.

Conclusion: Given it strong negative correlation with CD4 counts, an area under the curve of 0.99, specificity of 95.9% and sensitivity of 97.5%, neopterin may provide some prognostic information to CD4 counts used in the monitoring of patients infected with the HIV disease.

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ABBREVIATIONS

AIDS-Acquired Immunodeficiency Syndrome

ALT-Alanine Transaminase

ARF-Acute Renal Failure

ARV-Antiretroviral

AST-Aspartate Transaminase

AUC-Area under the curve

AZT-Zidovudine

BMI-Body mass index

BUN-Blood Urea Nitrogen

CRF-Circulating Recombinant Form

CDC-Center for Disease Control

CD4- Cluster of differentiation d4T

-Stavudine

EFV-Efavirenz

GHS-Ghana Health Service

HAART-Highly Active Antiretroviral Therapy

HIV-Immunodeficiency virus

HCT-Haematocrit

MCH-Mean Cell Haemoglobin

MCHC-Mean Cell Haemoglobin Concentration

MCV-Mean Cell Volume

MHC-Major Histocompatibility Complex

NNRTI-Non –Nucleoside Reverse Transcriptase Inhibitors

NRTI- Nucleoside Reverse Transcriptase Inhibitors PI-Protease Inhibitors

RBC-Red blood cell

RNA-Ribonucleic Acid

SIV-Simian Immunodeficiency Virus

SPSS-Statistical Package for Social Science

SQV-Saquinavir

TDF-Tenofovir

TLC-Total lymphocyte count

WBC-White blood cell

3TC-Lamivudine

CHAPTER 1 INTRODUCTION

1.1Background

Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS) has been a challenge to the medical fraternity in the last three decades due to the large mortality and morbidity caused by the devastating effects of the human immunodeficiency virus on the host's CD4 T lymphocyte. HIV infection has been described as a worldwide infection with the highest number of cases occurring in the sub-Saharan Africa (UNAIDS, 2002).

Initial infection with HIV is characterized by a symptomatic period where there are absent or low viral replication which result in a gradual reduction in CD4 cells and a total defect in the host's T cell function (El-Sadr *et al.*, 1987). The selective destruction of CD4 and T cell function consequently weakens the immune system and make patients susceptible to several clinical complications including haematological abnormalities, liver dysfunction, renal dysfunction, immunological complications, biochemical, metabolic complications and other opportunistic infections (Chu and Selwyn, 2011). These complications have been attributed to the major causes of death among patients who are infected with the disease (Moyle, 2001; Abrescia *et al.*, 2005).

Since the advent of the highly active antiretroviral therapy (HAART) which is based on the combination of nucleotide reverse transcriptase, non -nucleotide reverse transcriptase and protease or fusion inhibitors, they have been accepted worldwide as the gold standard treatment for individuals who are infected with the HIV (Odunukwe *et al.*, 2005). Although HAART has played a significant role in reducing mortality and morbidity thus dramatically increased life expectancy in HIV infected patients, a number of complications still exist due to the toxicity of the drugs which pose threats to the life of patients on the therapies. Some of these therapies have been associated

with nephrotoxicity, hepatotoxicity and other adverse effects including metabolic, gastrointestinal and central nervous toxicities (Carpenter *et al.*, 1998) but due to the benefit patients derive from the antiretroviral drugs, long time treatment is inevitable. In the light of these complications, management of the disease has been of public health concern considering the high potential risks to the patients (Cohan, 2000) .

Over the last few years several surrogate markers have been validated for monitoring of patients infected with the human immunodeficiency virus and their response to antiretroviral therapies. These markers are advantageous in that they are both relatively easy to quantify and predicts the clinical future of the patients (Lafeuillade *et al.*, 1994). Such markers including neopterin ,02microglobulin and other cellular markers produced at the early stages of the infection as a consequences of the host- cell interactions correlates closely with the viral RNA load in the serum and urine of the host cell (Wirleitner *et al.*, 2005) and they are particularly useful in the management of HIV infected individuals by aiding clinicians in the identification of patients who are at a greater risk of the disease progression.

1.2 Problem statement

The pathogenesis of the human immunodeficiency syndrome is characterized by a prolonged latent stage during which the virus continues to replicate leading to the depletion of the CD4 counts and weakening of the immune system which causes symptomatic illness (Miedema, 2006; Venkataramana, 2013).The progression of the disease is highly variable and hence the time for seroconversion varies from person to person. The biological basis of this variability in the disease progression is unknown but few studies have attributed it to defective virus (Venkataramana, 2013) which has been a challenge to the medical fraternity in the management of the HIV disease.

Evidence supports the premise of an associated adverse effect of the highly active antiretroviral therapy used in the management of HIV infected patients (Ejilemele *et al.*, 2007). This adverse effects evolving from mitochondrial dysfunction have been associated with several complications including biochemical, immunological and haematological abnormalities poses threats to the life of patients on these drugs (Abrescia *et al.*, 2005; Chu and Selwyn, 2011).

Several laboratory markers have been utilized for the monitoring of HIV infected individuals but the principal biological markers are the CD4 counts and the viral RNA load. Estimation of these markers are expensive and require considerable skills hence few laboratories in resource limited settings offer the test for patients making the clinical monitoring of patients difficult (Malone *et al.*, 1990; Balakrishnan *et al.*, 2005; Chadha *et al.*, 2013). In recent times one of the greatest challenges in the management and the care of HIV infected individuals in Ghana has been the evaluation of CD4 count measurement. There is therefore the need for cost effective, easily performed and readily available surrogate markers that can assist in predicting the disease progression and patient's response to HAART.

1.3 Justification

Although HAART has significantly improved the quality of life in HIV infected patients, long term use is limited by adverse side effect and drug resistance. The assessment of the impact of HAART has therefore become necessary in the management of people living with HIV infection. A major feature of HIV infection is the activation of all components of the immune system and an increased production of several surrogate cytokines which are indications of immunologic changes in the body during the HIV infection. However, the limitation in the ability to measure circulating cytokines has led to the determination of products of immune activation which reflect cytokine activity (Chandra *et al.*, 2005). Such assessment includes soluble markers and one such candidate

marker is neopterin. Serum neopterin is an indicator of immune suppression (Wirleitner *et al.*, 2005) and correlate with the disease progression, hence measuring serum neopterin will pave the way to better understand the immunopathogenesis of the HIV disease and can be used in predicting the clinical stage, initiation of HAART and the management of HIV disease especially in resource limited settings (Dar and Singh, 1999; Bipath *et al.*, 2015).

1.4 General objective

The main aim of the study is to evaluate serum neopterin as a marker of HIV disease progression and patient's response to antiretroviral therapy.

1.4.1 Specific objectives

1) To evaluate serum neopterin among HIV seropositive on HAART and HAART naïve HIV patients and also correlate serum neopterin levels with CD4 counts and other studied variables.
2) To compare some haematological parameters (Hb, RBC, WBC, MCH, MCV, HCT and MCHC) in HIV seropositive on HAART and HAART naïve patients so as to ascertain the trend in the disease progression.
3) To evaluate some biochemical parameters (albumin, total protein, globulin urea, creatinine, AST and ALT) in the disease progression among HIV seropositive on HAART and HAART naïve patients
4) To determine the association between BMI and CD4 count among HIV seropositive on HAART and HAART naïve patients.
5) To determine if neopterin, biochemical, haematological and body mass index predicts the progression/prognosis of HIV infection.

CHAPTER 2

LITERATURE REVIEW

2.1 HIV AND AIDS GLOBAL STATISTICS

In 2013, 35 million people were estimated to be living with the HIV infection which was a reduction compared to the 35.3 million in 2012. About 78 million people have been infected with the disease since the beginning of the epidemic and about 39 million people have died as a result of AIDS related illness. Globally AIDS related deaths have fallen by 35 % since the peak in 2005. There has also been a reduction in new infections among children to about 58%. The number of people living with the disease has increased in the last decade from 31.7 million in 2003 to 35.3 million in 2013 as a result of general population growth, continuing new infections and people living longer with the infection. However there has been a stabilisation in the epidemic due to the decline in new infections which has levelled the prevalence since 2001(USAID, 2014) .

2.1.1 HIV/AIDS Statistics in Ghana

HIV infections have declined drastically in the Saharan Africa between 2001 and 2010 with Ghana being among the top five countries whose national prevalence have declined to about 52% among people aged between 15-24 (USAID, 2011). In 2013 224, 488 people were estimated (189,931 adults and 34, 557 children) to be living with the HIV infection. New infections among children and adults were also estimated to be 7,812. There was also a drop in the prevalence among antenatal women to about 1.9% which was the first drop since the last decade. National prevalence in 2013 was 1.3% showing a decline from 3.6% in 2003. Eastern region had the highest regional prevalence of 3.7% with Northern and Upper East regions having the lowest regional prevalence of 0.8%. Sexual intercourse between the opposite sex accounted for about 75% to 85% of HIV infected cases, mother to child transmission accounted for 15% and transmission through blood and blood

products accounted for about 5% of HIV infected cases in Ghana (NACP, 2001). About 97.1% of all HIV infections are caused by HIV 1 whiles

HIV 2 accounts for only 0.8 % with 2.1% being caused by both HIV 1 and HIV 2 (GHS, 2004)

2.2 HISTORY AND ORIGIN OF HIV

In 1981 the center for disease control and prevention (CDC) reported the first cases of human immunodeficiency disease (HIV) after a biopsy taken from five young homosexuals confirmed pneumocystis pneumonia (Friedman-Kien *et al.*, 1981; Greene, 2007) . All these five homosexuals incidentally had a history of candidiasis and cytomegalovirus infection. These earlier reports were followed by series of pneumocystis pneumonia with Kaposi's sarcoma cases also in the male homosexuals (Greene, 2007). The cause of this highly devastating illness remained unknown until 1983 when the retrovirus called lymphadenopathy-associated virus

(LAV) which was isolated by Luc Montagnier infected and killed CD4 cells (Barre-Sinoussi, 1983). In 1994 the human T-lymphotrophic virus (HTLV-III) was identified (Gallo *et al.*, 1984) but both viruses were later renamed HIV because they had the same genetic composition (Chang *et al.*, 1993). There have been several explanations as to the origin of HIV. Sharp *et al.* (1994) made a speculation that the HIV originated in non-human primates in sub- immunodeficiency virus which was found in the sooty mangabey .Several alternative explanations for the origin of AIDS have been put forward with the argument that the causative organism of AIDS is not HIV and that the disease does not exist. Others have associated AIDS to be caused by an individual's way of life including drug usage and one's sexual life and some argue that the virus was generated in the US primate laboratory center through the inoculation of tissues from sooty mangabeys who were naturally infected into health macaques species (Apetrei *et al.*, 2005) , but all these hypotheses have been disproved and rejected by scientific studies.

2.3 CLASSIFICATION OF HIV

The human immunodeficiency virus is a member of group of viruses known as retroviruses and are further classified into the subgroup of viruses called lentivirus which includes SIV and feline immunodeficiency virus (FIV). Retroviruses are exceptional group of viruses because their genes are located on the RNA whereas most viruses have their genetic materials located along strands of DNA. HIV has many different strains because of the rapid mutation of the virus. On the basis of their similarities, these numerous strains have been further grouped into types and subtypes (Plantier *et al.*, 2009)

2.3.1 Types of HIV

Two types of HIV namely HIV-1 and HIV-2 have been identified. The HIV-1 which was identified by Luc Montagnier in 1983 is more virulent compared to HIV-2 which was discovered among Cameroonian patients in 1985. HIV-1 is more similar to the simian immunodeficiency virus in the chimpanzee whiles HIV-2 is more related to the sooty mangabey. HIV-2 is less abundant with majority of its infections occurring in West Africa compared to HIV-1 which is more abundant with its infections occurring worldwide (Plantier *et al.*, 2009).

2.3.2 Human Immunodeficiency Virus 1

Four groups of HIV 1 namely M, N, O and P have been identified. Group O is restricted to West Central Africa whiles group N is extremely uncommon type which was first identified in 1998 in Cameroon (Plantier *et al.*, 2009) .Group P which was identified in 2009 is more similar to gorilla simian immunodeficiency virus (Plantier *et al.*, 2009) .Group M is the most abundant with nine subtypes (A, B, C, D, F, G, H J and K) and accounts for about 90% of all infections caused by

HIV-1. Combination of different subtypes leads to the exchange of genetic information which produces a new strain of virus in a process unknown as viral sex (Burke, 1997).

Group	
• Subtype A	West and Central Africa
• Subtype B	South America (including Brazil and Argentina), United States, Europe, Thailand, Russia
• Subtype C	India, Sudan, Southern and Eastern Africa
• Subtype D	East and Central Africa
• Subtype E	Thailand, Philippines, China, Central Africa
• Subtype F	Brazil, Argentina, Eastern Europe, Central Africa
• Subtype G	Western and Eastern Africa, Central Europe
• Subtype H	Central Africa
• Subtype J	Central America
• Subtype K	Democratic Republic of Congo, Cameroon
• **N**	Cameroon
• **O**	West Africa

Table 2.1 Distribution and subtypes of HIV-1

2.3.3 Human Immunodeficiency Virus 2

HIV-2 is mainly restricted to West Africa with Senegal and Guinea Bissau being documented as the regions with the highest infection rate of HIV-2 since its discovery (de Silva *et al.*, 2008). In most West African countries the prevalence of HIV-2 have declined with majority of the infections being caused by HIV-1 (Hamel *et al.*, 2007). The viral burden in HIV-2 infections are relatively lower than infections caused by HIV-1 which possibly explains the reduced rate of transmission and total absence of mother to child transmissions of HIV-2 (Berry *et al.*, 2002). Although infections caused

by HIV-2 does not usually results into AIDS, individuals who progress to AIDS show symptoms which are similar to infections caused by HIV-1 (RowlandJones and Whittle, 2007). Thus it is indicative that HIV-2 infection differs appreciably from
HIV- 1 with respect to their natural history which is not quite surprising, given the fact that HIV-2 was derived from a primate lentivirus different from that of HIV- 1.

2.3.3.1 Subtypes of HIV 2

Since its isolation eight different subtypes have been discovered with each having a distinct host transfer. These subtypes have been named from group A to H but only groups A and B have spread appreciably among humans (Sharp and Hahn, 2011). Group A is mostly restricted to western Africa (Peeters *et al.*, 2003) whereas group B is predominantly found in Cote d'Ivoire (Ishikawa *et al.*, 2001). Recently another strain of HIV-2 (group F) have been found to be associated with a rapid decline in CD4 count and a higher viral loads (Smith *et al.*, 2008).

2.3.3.2 HIV 2 mode of transmission

The mode of transmission of HIV-2 is similar to that of HIV-1, however commercial sex workers and people with other sexually transmitted diseases have a higher risk of being infected. Infection rate among males and females appears to be the same but people infected with HIV-2 are usually older than individuals infected with HIV-1(Miyazaki, 1995).The cellular mechanisms (CD4 receptors and chemokine coreceptors) utilised for establishing an infection are the same for both HIV-1 and HIV-2 (Murdoch and Finn, 2000) .

2.4 THE STRUCTURAL ORGANISATION OF HIV

HIV exists as roughly spherical particles with a diameter of about 120 nm. It is surrounded by a viral envelope which is made up of lipoprotein materials. There are about 72 spikes which project

from the viral envelope and form the gp 41 and gp120 proteins. The viral matrix which is formed from the protein gp 17 is found just below the viral envelope. The viral proteins gp 120 and gp17 forms the spikes on the outer part of the virus whiles the viral core and matrix are formed from gp 17 and gp 24 respectively. The viral core reveals two identical stands of RNA which are the genetic materials of the virus. Located in the viral core are also reverse transcriptase, integrase and protease which are the three enzymes involved in the viral replication

(Freed and Martin, 1995).

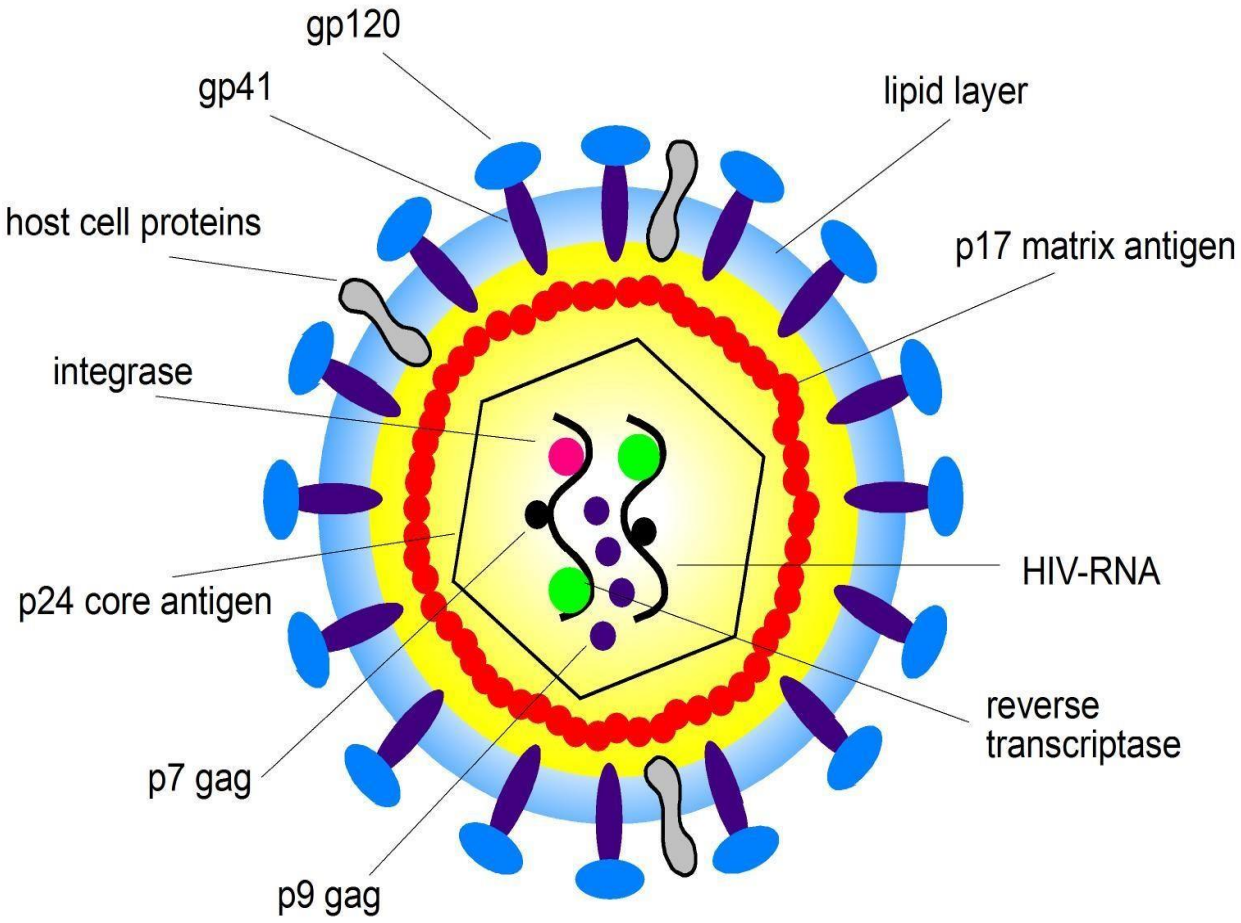

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Figure 2.1 A diagram showing the structure of HIV

2.4.1 The genomic structure of HIV

The genomic structure of the viral particle reveals three structural genes (gag gene, env gene and pol gene) and other six genes which have been classified into two groups namely accessory and regulatory genes.

2.4.1.1 Structural Genes

Retroviruses are composed of three structural genes namely the envelope genes, polymerase genes and the group antigen genes. The envelope genes (env gene) are involved in the production of the viral envelope and other glycoproteins such as gp 41 and gp 120 which are found on the spikes that project from the outer layer of the virus whiles the polymerase genes are involved (pol gene) in the production of the viral inner core proteins (Greene, 1991).The group antigen genes code for the production of reverse transcriptase and viral enzymes such integrase and protease which are involved in the replication of the virus (Schoub, 1999).

2.4.1.2 Regulatory Genes

The regulatory genes are tat and rev genes. These genes assemble and bind to defined regions of the viral RNA within the nucleus. The Tat gene (transactivating protein) is the major protein involved in the upregulation of the viral replication. The tat genes together with other cellular proteins bind to specific site on the long terminal repeats and promote the transcription of messenger RNA for the production of viral proteins. Rev genes (regulator of expression of viral proteins) are involved in exporting of viral RNA from the nucleus of the cell (Schoub, 1999)

2.4.1.3 Accessory Genes

Four genes namely virus infectivity factor (vif), viral protein r (vpr), viral protein u (vpu) and negative factor (nef) have been classified as accessory genes because they are not directly involved in the viral replication. Vif influences the ability of the virus to establish the infection, enhances horizontal transmission and releases infectious virus from the cell. Nef protein inhibits the production of structural proteins by producing proteins known as the negative regulatory element which act on the long terminal repeat thereby down regulating viral replication. The Vpu are involved in the deterioration of the cluster of differentiation cells (CD4) and impairs its functions. They also enhance the release of virions from the surface of infected cells. Vpr are also involved in the induction of cellular differentiation and enhances the ability of the virus to infect cells by promoting the import of preintegration complexes from the nucleus (Schoub, 1999).

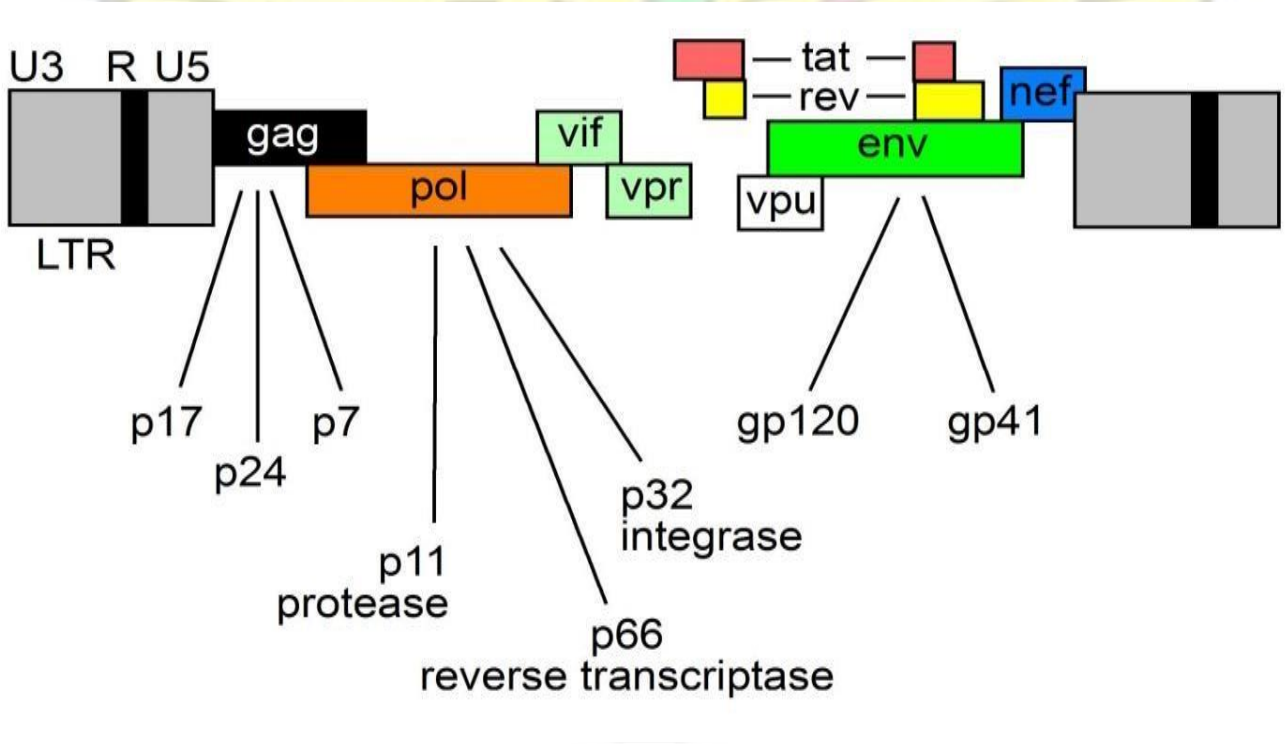


Figure 2 2 A diagram showing the organisation of the viral genome

2.5 VIRAL REPLICATION

The HIV life cycle includes the following stages; binding and entry, reverse transcription, integration, replication, budding and maturation.

2.5.1 Entry and Binding

Entry of viruses into the host cell is facilitated by the viral proteins gp41 and gp 120 which are found on the spikes of the viral envelope. When a viral particle come into contact with a host cell, the spikes on the outer part of the virus are fused to the CD4 receptor of the host cell allowing entry of the virus into the host cell's membrane. The virus then releases its content into the host cell leaving the viral envelope outside the host cell (Azevedo-Pereira *et al.*, 2005)

2.5.2 Transcription and Integration

Within the host cell, there is the conversion of the viral RNA to DNA by the enzyme known as reverse transcriptase so as to make the viral particle compatible to the genetic material of the host cell. The newly single stranded viral DNA undergoes replication into double stranded viral DNA. It is then transported to the nucleus of the host cell and becomes inserted into the host cell's DNA by the enzyme known as the viral integrase.

2.5.3 Transcription and Translation

The newly produced viral particle may be adamant for some time until the cell becomes activated. Upon activation of the cell, there is the conversion of the viral genes into messenger RNA which is then transported into the cytoplasm of the host cell and then used for the production of new viral proteins and enzymes (Emerman and Malim, 1998).

2.5.4 Assemble, Budding and Maturation

During this stage, the enzyme protease cut the long strands of viral proteins into smaller proteins with some forming viral enzymes and others forming the structural element of the virus. These newly produced viral enzymes, proteins and the messenger RNA come together and bud out of the host cell to form new viral particle .After the maturation period, these viruses are now capable of infecting new cells (Emerman and Malim, 1998)

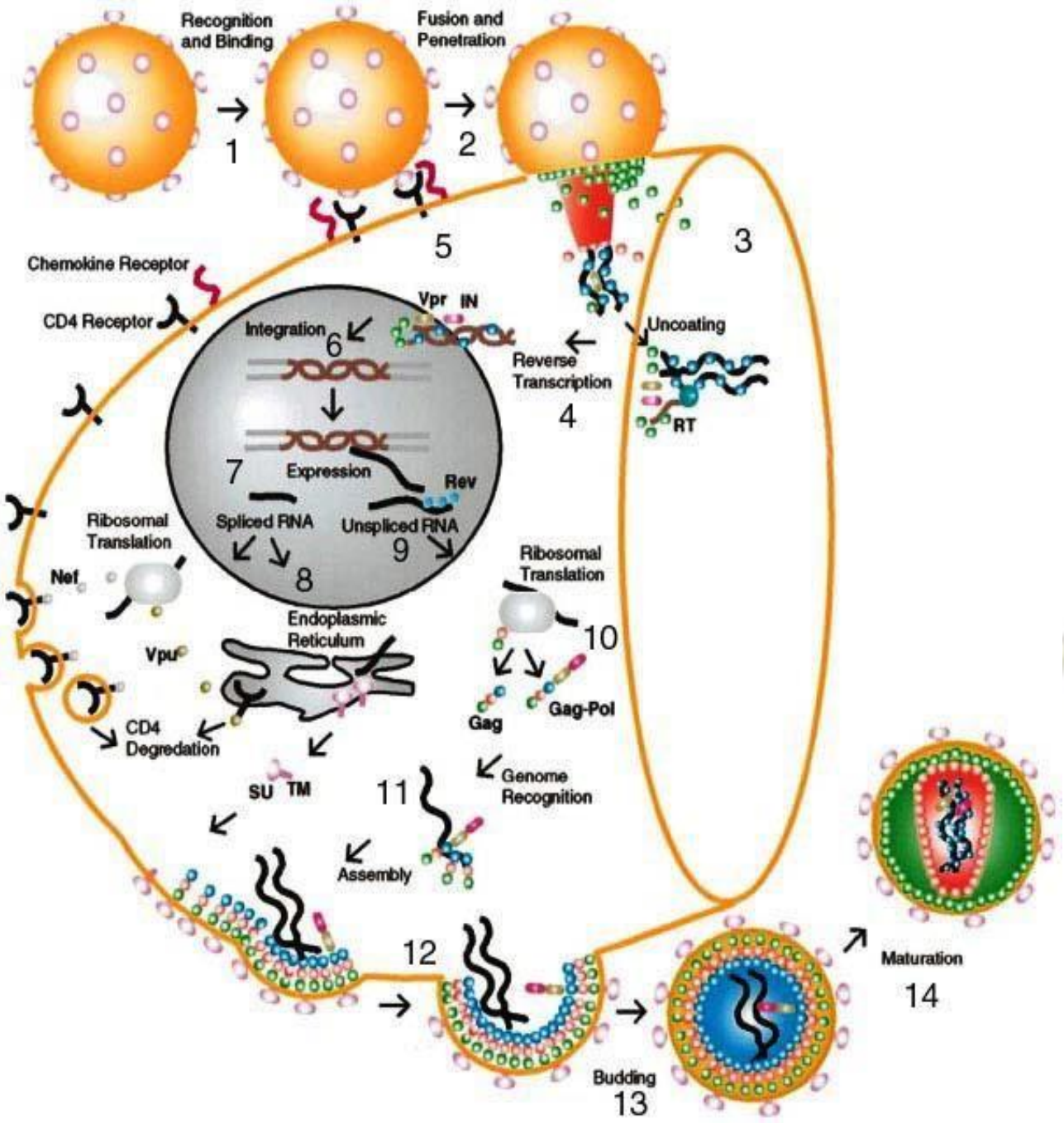


Figure 2.3 Replicative cycle of HIV(Emerman and Malim, 1998)

2.6 NATURAL COURSE OF HIV INFECTION

The disease progression differs significantly among people infected with HIV. Some individuals develop AIDS within eight to ten years after seroconversion and these people are known as the typical progressors. Development of AIDS may also take about two to five after seroconversion whiles others may be asymptomatic for about ten years after seroconversion (late progressors) (Levy, 2007).The natural course of HIV infection are classified as; acute/primary infection, clinical latency and AIDS stage.

2.6.1 Acute phase of HIV Infection

During this phase some patients remain asymptomatic but majority show symptoms including fever, sore throat, skin rash and nausea (Schacker *et al.*, 1996). Viral replication peaks during this phase resulting in the decline of the CD4 cells. As part of the host immune response to the infection, the cytotoxic T cells stimulate the elimination and death of cells and other tissues which expresses the virus (virus –expressing cells). Specific HIV antibodies are also produced which contributes to the decline in the level of virus in the host cell (Clark *et al.*, 1991).

2.6.2 Latent phase

This phase is characterised by a temporary rise in CD4 counts and a decline in the level of virus present in the host cell. Because the CD4 lymphocytes cannot completely regenerate there is a persistent decline in the functions of the immune cells thereby enhancing the replication of the virus. Following a decline in the level of the CD4 count below $200mm^{-3}$ the host immune cell loses the ability to control the virus making patients more susceptible to opportunistic infections (Levy, 2007).

2.6.3 The AIDS Stage

This stage is characterised by the appearance of opportunistic infections or neoplasms. AIDS progression is also marked by the formation of syncytium variant. These syncytia-forming (SI) variants have a greater specificity for CD4+ cell which enhances a more rapid decline in the CD4+ cell. Although before the onset of the AIDs stage, there is a rise in SI variants when the CD4 count is between 400 and 500/µL, it has been shown to be a prognostic marker that is independent of the CD4 counts (Ghose *et al.*, 2001).

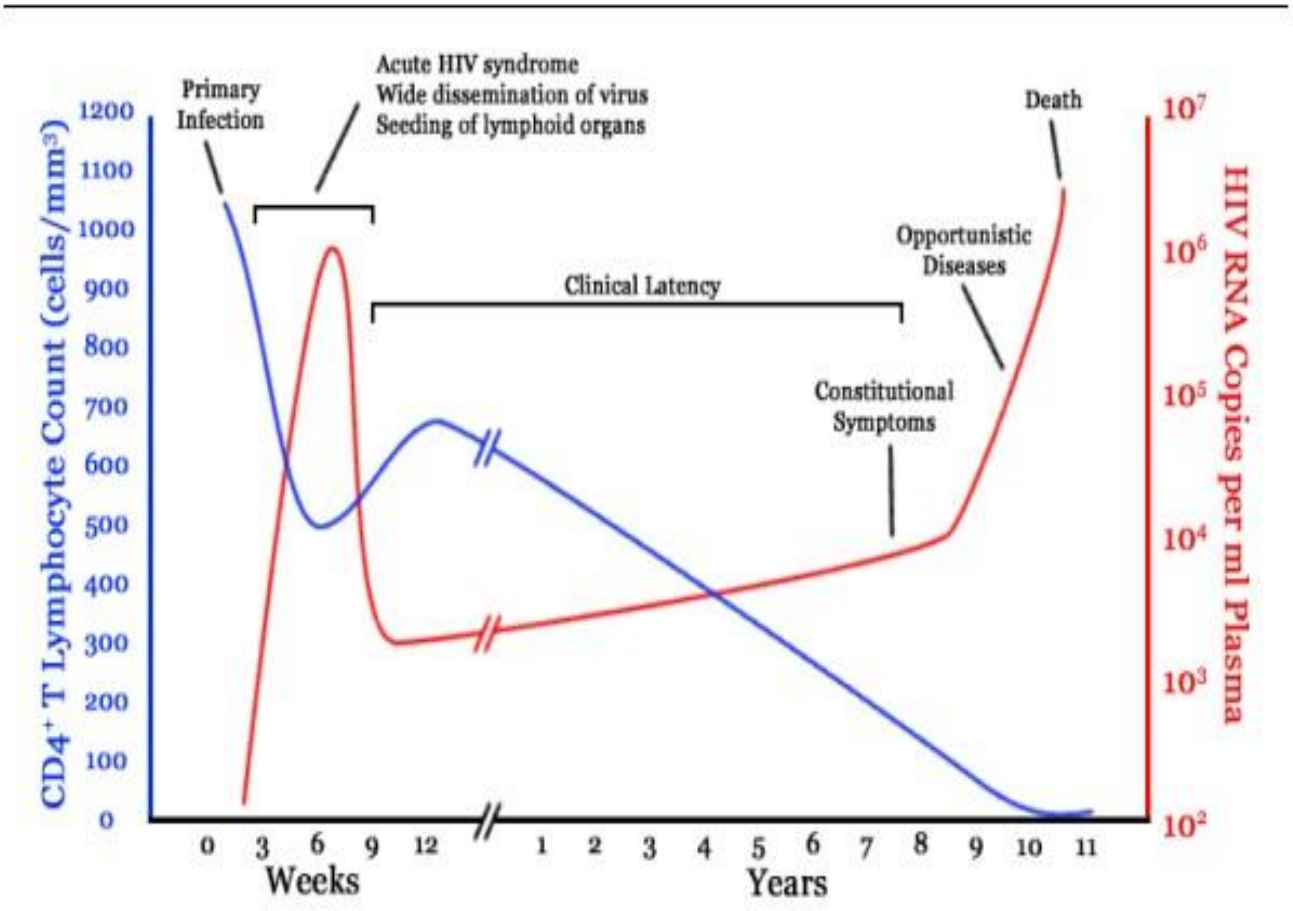


Figure 2.4 Natural course of HIV infection (Fauci *et al.*, 1996)

2.7 IMMUNE RESPONSE TO HIV

The immune response elicited during HIV infection by the host is largely determined by the CD4 T lymphocyte cells of the T helper 1 cells. Initial infection with HIV is followed by the emergence of CD8+ cytotoxic T cell responses which bind to the viral peptide presented by the major histocompatibility complex class 1(MHC1) that are located on the surface of the infected cell. This results in the destruction of the target cell producing the virus by triggering a cytotoxic response thereby decreasing the plasma levels of the virus (Koup *et al.*, 1994). A number of cytokines including gamma interferon are also released by the CD8 cytotoxic T cells which have been shown to have antiviral activities. (Frucht *et al.*, 2001). These responses during the HIV infection have been shown to decrease with the emergence of the production of antibodies by the T helper 2 cells and this shift from T helper 1 response to T helper 2 responses have been largely attributed to the development of AIDS .Earlier reports (Marone *et al.*, 2001) have indicated that the shift in TH response from THI to TH 2 is caused by the triggering of the HIV associated cytokines mediated by the gp 20 and the tat genes.

2.8 ANTIRETROVIRAL DRUGS IN HIV MANAGEMENT

In 1986, antiretroviral drugs (ARVs) were introduced for the treatment of HIV infection which involved combination of several such drugs termed highly active antiretroviral therapy (HAART) (Bartlett *et al.*, 2004).These drugs have been the gold standard treatment for individuals infected with the disease but due to the severity of the adverse effects, difficulty in selecting and following a regimen, and the importance of adherence to prevent viral resistance, emphasis of involving patients in therapy choices is very crucial (Dybul *et al.*, 2002).

2.8.1 Classes of Antiretroviral therapy

There are various classes of antiretroviral drugs that work to inhibit the life cycle of HIV at different stages. These include nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside/ nucleotide reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), integrase Inhibitors and fusion inhibitors (FIs).

2.8.1.1 Nucleotide and Nucleoside Reverse Transcriptase Inhibitors (NRTIs)

These drugs acts against the enzymes reverse transcriptase by incorporating itself into the viral DNA. This prevents its elongation and terminates the process of transcription. Zidovudine (AZT), stavudine (d4T), lamivudine (3TC) and emtricitabine are examples of drugs in this class.

2.8.1.2 Non Nucleotide/Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)

These drugs bind to active sites on the reverse transcriptase enzyme thereby inhibiting its functions directly. Efavirenz and Nevirapine are examples of drugs in this class.

2.7.1.3 Protease Inhibitors (PIs)

Protease inhibitors act against protease thereby preventing the cleavage of nascent viral proteins for final assemblage of newly produced viral particles. Nelfinavir, Indinavir and saquinavir are some examples of protease inhibitors (de Soultrait *et al.*, 2002).

2.7.1.4 Integrase Inhibitors

These drugs work to inhibit the viral integrase enzyme and prevent the viral DNA from being integrated into the DNA of the host cell. Raltegravir is an example of an integrase inhibitor (de Soultrait *et al.*, 2002).

2.7.1.5 Entry and Fusion Inhibitors

These drugs block the entry targets of the virus which helps to halt the infection by preventing entry, binding and fusion of the virus into the host cell. Currently, Enfuvirtide and Maraviroc are the two registered drugs in this class (Kilby *et al.*, 1998).

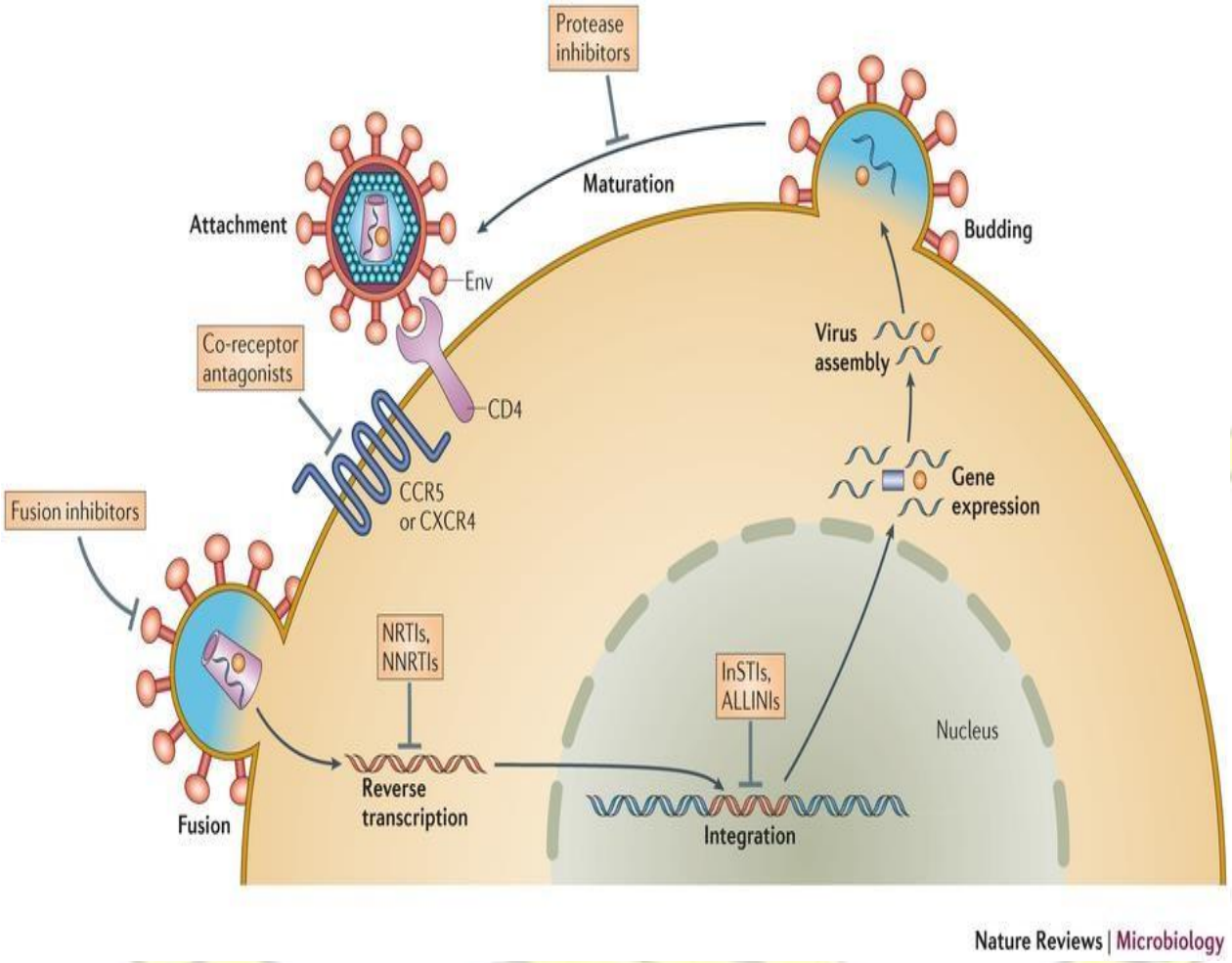


Figure 2.5 Targets of HAART in HIV replicative cycle

2.8.2 Combination of ARV therapy

The replicative cycle of HIV from viral entry to maturation stage can last for about 1.5 days

(Perelson *et al.*, 1996). Due to the absence of proofreading enzymes in the HIV, errors made during the conversion of viral DNA into RNA are not corrected which enhances rapid mutation of the virus. This coupled with the short life cycle results in high genetic variability of the virus. Combination of antiretroviral therapy has therefore become important in the management of HIV patients in this HAART era given the fact that the rapid viral mutation may cause HAART patients to develop drug resistance. Antiretroviral combinations inhibit viral replication which keep the number of virions low and reduces the likelihood of changing the genetic composition of the virus. Except in few cases, no single antiretroviral therapy has shown to inhibit HIV infection for long hence these therapies should be combined in order to attain a long lasting benefit. Usually these therapies comprises of two NRTIs and one NNRTIs or one PIs (Bartlett *et al.*, 2004). Although these combinations are useful, treatment options of some patients can be limited by the complicated dosages schedules and other undesirable side effects. Hence pharmaceutical industries in recent time have made fixed dosage formulation drugs by combinations of the complex antiretroviral therapies. This has greatly improved the difficulty in taking the drugs thereby enhancing the efficacy of the drugs. Patients can now maintain one regimen for years without developing resistance depending on their high adherence rate which increases their chances of long-term survival.

2.8.3 HAART Initiation

There are several treatment guidelines for antiretroviral therapy (ART). For resource limited settings, World Health Organization (WHO) recommends that individuals confirmed to be HIV seropositive should be initiated onto the therapy when one of these conditions is evident;

- HIV infected individuals with WHO stage three or four or individuals with CD4 count less than 500mm^{-3} should be placed on the therapy.
- HIV infected individuals with other coinfection such tuberculosis and chronic liver disease should be initiated onto the therapy.
- HAART should also be initiated for HIV seropositive pregnant and breast feeding women (WHO, 2013)

2.8.4 Mechanism of HAART Toxicity

NRTIs impairs the synthesis of mitochondrial deoxyribonucleic acid by binding to deoxyribonucleic acid polymerase gamma which is the enzyme involved in the replication of the mitochondrial DNA. This may lead to the disruption in respiration which could results in apoptosis (programmed cell death). NRTIs are also involved in liver injury through other mitochondrial toxicities such as hepatic steatosis, lactic acidosis and lipoatrophy. NRTIs toxicity to the mitochondrion differs significantly among the various nucleotide classes with zalcitabine having the highest toxicity, didanosine, stavudine, zidovudine, abacavir and tenofivir as least toxic respectively (Birkus *et al.*, 2002; Verucchi *et al.*, 2003). Among the NNRTIs, necrosis of the hepatic cell have been associated with the usage of nevirapine especially in patients with CD4 counts greater than 250 mm^{-3} whiles the likelihood of developing liver enzyme elevation have also been linked to the usage of efavirenz (Rivero *et al.*, 2007). Protease inhibitors have been shown to cause elevation of liver enzyme. Ritonavir which is hardly used in clinical settings has the highest risk of liver toxicity (Sulkowski *et al.*, 2000).

2.9 MARKERS OF HIV DISEASE PROGRESSION

The pathogenesis of HIV infection results in a series of interactions between the virus and the host cell leading to continuous activation of the immune system. The extent of the immune activation can be used to monitor the HIV disease progression by determining serum levels of markers such as neopterin and 02 microglobulin and other HIV cellular markers which are products of immune activation (Fahey *et al.*, 1990). The use of nonclinical markers are very important in the management of patients in that the development of AIDS after seroconversion varies from person to person (Dar and Singh, 1999). Due to the high production rate of viruses at the different phases of the infection, measuring of viral RNA loads or features which reflects activites of the virus has been of importance in establishing the exact relationship between the virus and the level of immune activation, progression of the disease , replication rate of the virus and the estimated duration that patients may become resistant to the antiretroviral therapy (Dar and Singh, 1999). At the early stages of the infection, it provides an information on the course the infection is likely to take and can be used as a basis for appropriate treatment decisions. Viral load measurement is useful in acertaining the efficacy of a specific antiretroviral therapy during treatment (Saag *et al.*, 1996). Besides measuring the viral RNA loads, other cellular markers such as CD4 counts have been widely used by clinicians in monitoring HIV dissease progression and a higher HIV RNA levels have been shown to correlate with lower baseline CD4+ T-cell counts and a rapid progression of the disease (Saag *et al.*, 1996).

Type of Marker (Activation)	Mechanism (Host-dependent)
Cellular Markers	HLA-DR+ IL-2R+ T Cells
Soluble Markers	ß -M 2 neopterin sIL-2R sCD4 sCD8
Antibody Production	anti-gp120 anti-p24 IgA

(Tsoukas and Bernard, 1994)

Table 2.2 Early markers of HIV disease progression

Type of Marker (Immune Dysfunction)	Mechanism (Virus-dependent)
Cellular Depletion	$CD4^+$ T Cells
Cytokine Depletion	IFN IL-2
Antibody Depletion	anti-p24 anti-gp120

(Tsoukas and Bernard, 1994)

Table 2.3 Late markers of HIV disease progression

2.9.1 BODY MASS INDEX AS A MARKER OF HIV DISEASE PROGRESSION

The measurement of body mass index in HIV disease has been of importance in the management of people who are infected with the disease due to the presence of wasting syndrome which is a common occurrence during the HIV infection (Palella Jr *et al.*, 1998). Several studies have found BMI to correlate with the disease progression and patient's response to antiretroviral therapy (Jones *et al.*, 2003; Langford *et al.*, 2007). In an observational cohort study aimed at assessing the relationship between BMI and CD4 before and after the treatment with HAART, Koethe *et al.* (2011) found CD4 counts to be increased among patients whose pretreatment BMI was between 25-30 kg/m^2 and decreased among patients whose BMI was above and below this range and that BMI correlate with immune constitution and a slower progression to AIDS .Denue *et al.* (2013) also found a positive relationship between BMI and CD4 counts after six months of being on the HAART therapy and that a higher BMI may be associated with an improved immune reconstitution. According to Calle *et al.* (2003) and Shuter *et al.* (2001) although overweight and

obese may be associated to various metabolic complications irrespective of HIV infection, increased BMI during HIV infection have been shown to decrease the disease progression of HIV related mortality and morbidity.

2.9.2 NEOPTERIN AS A MARKER OF HIV

Neopterin is derived from the metabolism of guanosine triphosphate by macrophages and dendritic cells upon activation of gamma interferon via guanosine triphosphate cyclohydrolase-1 enzyme as shown in figure 2.6 below. It is therefore considered as a marker for cellular immune activation (Huber *et al.*, 1984; Nyamweya *et al.*, 2012) which is found in the human body fluids during times of cellular immune responses, for example during viral, bacterial and parasitic infections, and in autoimmune disease, malignancy and allograft rejection (Wirleitner *et al.*, 2005).

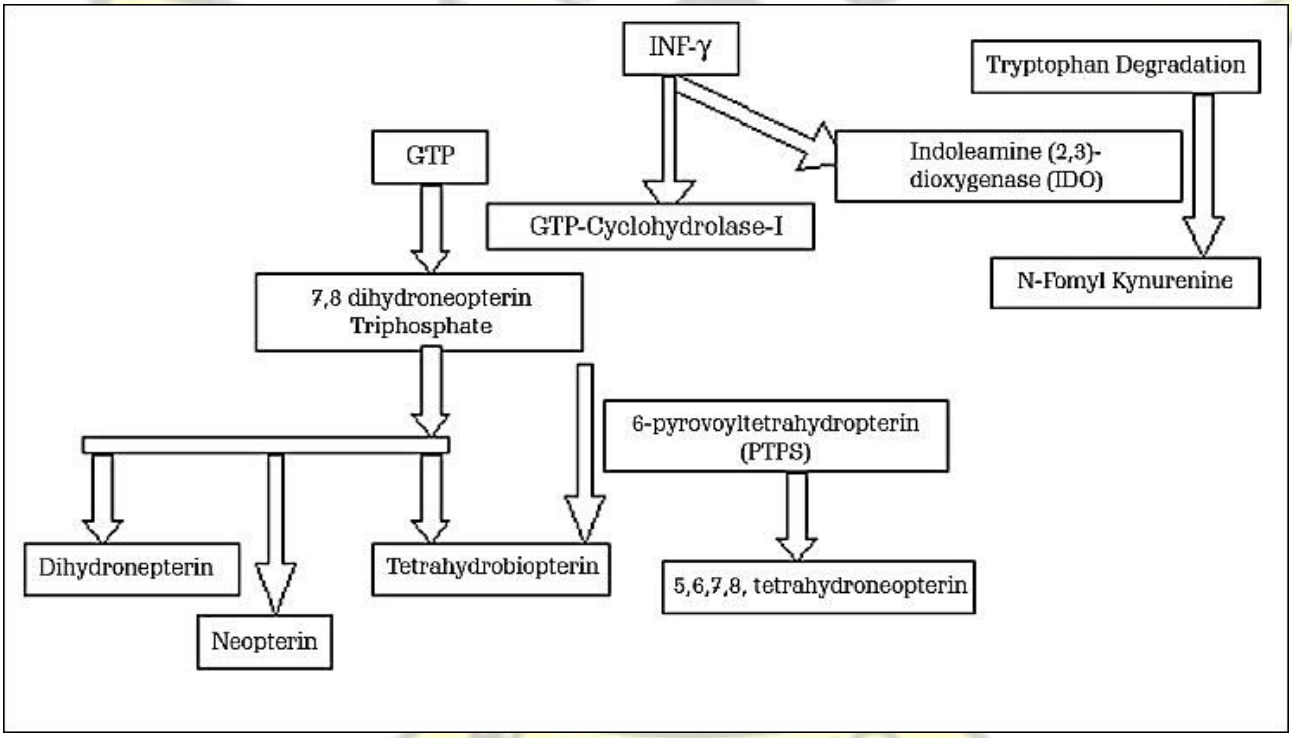


Figure 2.6 Mechanism of neopterin synthesis

2.9.2.1 Neopterin in HIV infection

Increased neopterin levels can be found in the serum and urine of almost all HIV patients (Fuchs *et al.*, 1989). Before the seroconversion of HIV, neopterin levels in HIV infected individuals does not differ significantly from that found in other viral infections except that levels of serum neopterin returns to the normal in other viral infections whereas levels continue to rise after HIVseroconversion (Fahey *et al.*, 1990; Zangerle *et al.*, 1992). Serum neopterin levels have been shown to be increased during the early stage of the HIV infection where patients shows no signs and symptoms of the infection and levels continue to rise as the disease progresses with highest levels showing in individuals with CD4 counts less than $200mm^{-3}$(Mildvan *et al.*, 2005a; Chadha *et al.*, 2013). This early increase in serum neopterin in asymptomatic individuals preceeds CD4+T-cell decline (Fahey *et al.*, 1990) , correlate with plasma HIV viral load and also predict HIV related mortality (Nyamweya *et al.*, 2012). Neopterin values therefore indicates the immunological status of individuals infected with the disease and may also reflects activities of cytokines which are invovled in the pathogensis of the HIV infection. Hence, initial values may be useful in predicting the course of the infection and also to identify individuals who are at a greater risk of the disease progression (Huber *et al.*, 1984; Nyamweya *et al.*, 2012).

2.9.2.2 Effect of HAART on neopterin

According Palella Jr *et al.* (1998), the highly active antiretroviral therapy which constitute two reverse transcriptase inhibitors and one protease inhibitor has significantly led to the reduction in viral load thereby restoring the quality of life ,prolonging survival and a decline in mortality and morbidity among HIV infected individuals. Several mechanisms have been proposed as to the

effect of these therapies on the serum neopterin levels of patients infected with HIV. In a randomized doubled blind study aimed at evaluating the effect of HAART treatment on the human immunodeficiency virus, Collier *et al.* (1996) found HAART to be associated with an increase in the CD4 T lymphocyte cells and a decline in markers of immune activation which promotes a partial recovery in functions of the immune cells. In another cross sectional study, the functions of the lymphoid progenitor cells including the differentiation into the B and T lymphocytes have also been shown to normalize among HIV patients treated with the highly active antiretroviral therapy (Nielsen *et al.*, 1998). HAART has been shown to induce changes in the T helper 1 and T helper 2 which differentiate to produce cytotoxic T cells and antibodies respectively (Pakker *et al.*, 1998) and also reverses the defects in the CD4 cells (Autran *et al.*, 1997). The cumulative effect of these mechanisms of HAART on the immune cells is the restoration of interleukin 2 productions which modulate various aspect of the immune response (Daniel *et al.*, 1999) and a decline in the level of cytokines involved in the pathogenesis of HIV infection. Consequently there is a reduction in the immune activation and the circulating levels of serum neopterin in HIV infected individuals (Wirleitner *et al.*, 2005).

Several studies have confirmed a decline in serum neopterin level among HIV infected individuals who have been treated with the highly active antiretroviral therapy (Mildvan *et al.*, 2005b; Wirleitner *et al.*, 2005). In a study aimed at evaluating the impact of HAART on circulating levels of immune markers among HIV infected individuals, Amirayan-Chevillard *et al.* (2000) observed a decline in serum neopterin to about 30% among patients treated with the HAART (61.7 nmol/L) compared to their HAART naïve patients (88.1 nmol/L). Chadha *et al.* (2013) also observed a decrease in serum neopterin to about 70% among patients who have been treated with HAART for a period of six months (from 38.6 nmol/L to 11.2 nmol/L).

2.10 COMPLICATIONS OF HIV INFECTION

HIV infection weakens the immune system and make patients susceptible to several clinical complications including haematological abnormalities, liver dysfunction, renal dysfunction, immunological complications, biochemical and metabolic complications (Chu and Selwyn, 2011).

2.10.1 Immunological Complications

HIV infection is associated with several effects on the immune system of the host. There is a decline in B cell functions (Patke and Shearer, 2000) and a decrease in the number of the CD4 counts which results from the distortions in regulations of cytokines (Margolick *et al.*, 1998). Asymptomatic individuals show distinct responses to the infection compared to individuals who do not exhibit symptoms. There are more T helper-1 cytokines such as interferon and interleukin-2 in individuals who do not progress faster with the disease (typical non progressors) contributing to an increase in the activities of the cytotoxic T cells and the CD4 T lymphocyte cells. Owing to this, the viruses also vary their antigenic site which enables them to escape the host immune defenses (Borrow *et al.*, 1997). The viruses may also counter the host immune defenses by producing major histocompatibility complex on the surfaces of their cells resulting in the decline in cytotoxic T cells functions (Pantaleo *et al.*, 1997).

2.10.2 Biochemical Complications

Infection with HIV stimulates an acute phase response and as a result the plasma concentrations of a number of proteins are changed in HIV patients. Fleck (1989) observed distortions in total protein productions to be associated with cytokine synthesis, leukocyte proliferation and the production of immunoglobulins. Several other mechanisms including inflammations and permeability of the capillaries have also been suggested to cause a decrease in proteins among HIV infected

individuals but returns to normal after resolution of the inflammation (Fuhrman *et al.*, 2004; Banh, 2006). Hypoalbuminemia have also been shown to predominant in patients with advanced stage of the disease due to a deficit in the hepatic synthesis against its catabolism which is caused by the HIV infection (Fleck, 1989). Globulin levels have also been shown to be elevated during HIV infection especially in patients who have advanced with the disease (Moir and Fauci, 2009).

2.10.3 Liver Dysfunction

Liver enzyme elevations are common disorders associated with HIV infection. In some cases, elevation of hepatic enzymes in HIV infected patients could be secondary to factors such as alcohol consumption, co-infection such as hepatitis B or hepatitis C infection (Mata-Marín *et al.*, 2009). The genesis of elevated liver enzymes in HIV infected individuals has been attributed to the toxicity of the antiretroviral therapy (Ngala *et al.*, 2015) whiles other researchers have also attributed it to the HIV infection itself (Ayelagbe *et al.*, 2014; Osakunor *et al.*, 2015a) but due to the benefit HIV patients derive from the antiretroviral treatment including immunological stabilization long-term treatment is inevitable (Hammer *et al.*, 1997). Long term hepatic damage may also result in mitochondrial toxicity, alteration in lipid metabolism and insulin resistance which contribute to the development of steatohepatitis and steatosis (Sulkowski *et al.*, 2005). These liver damages resulting from the drug toxicities coupled with drug discontinuation have been shown be a major cause of death in HIV infected (Ejilemele *et al.*, 2007) .Hence in this HAART combination era, major issues regarding the management and prevention of HAART induced liver injuries have evolve as public health concern (Palella Jr *et al.*, 2006).

2.10.4 Renal Dysfunction

Renal dysfunction during HIV infection have been shown to be a common disorder among individuals living with the infection (Szczech *et al.*, 2002).Wyatt *et al.* (2007) have showed that

15.5% of HIV infected patients develop chronic or end-stage renal disease. The disturbances in renal and tubular dysfunction predispose patients infected with HIV to haemodynamic and renal injuries. HIV-associated glomerulopathies and acute renal failure (Sarfo *et al.*) are examples of clinical syndromes that can result in toxic renal injuries (Rao, 1998) .HIV infected subjects have shown to be more likely to develop acute renal function than similarly matched subjects not infected with HIV(Valeri and Neusy, 1991; Rao, 1996) .In HIV infected patients, nephrotoxic and ischemic acute tubular necrosis, crystal induced tubular injuries, obstructive uropathy and pre-renal azotemia are the contributing factors of acute renal function (Rao, 1998).

2.10.5 Haematological Complications

A number of haematological manifestations are seen in the course of HIV infection which poses a challenge in the management of the disease. According to Moyle (2001) haematological complications including anaemia ,lymphopoenia, thrombocytopoenia and neutropoenia are the second most common cause of HIV related mortality and morbidity in people who are infected with the disease. Several mechanisms have been put across as to the exact causes of these complications in HIV infected individuals. Some researchers have attributed it to cytokine involvement in myelosuppression as the viral burden increases (Obirikorang and Yeboah, 2009) whiles others have attributed it to the toxicity of the antiretroviral therapy (Mildvan, 2003). Cytopoenia during HIV infection have been shown to correlate with the progression of the disease with anaemia accounting for about 70% of all HIV related haematological disorders (Volberding, 2002; Odunukwe *et al.*, 2005) .The mechanism of anaemia in HIV infection may be due to loss of blood which is linked to several conditions including neoplastic disease that affects the alimentary tracts and other damages of the alimentary canal that is caused by infections with cytomegalovirus. Three mechanisms including ineffective red blood cell production, increased red blood cell

destructions and decreased red blood cell production have been put forward to be involved in the pathophysiology of anaemia other than blood loses (Volberding, 2002). Inefficient production of red blood cell (RBC) may be caused by a deficit in certain food nutrients such foliate, iron and vitamin B 12 whiles decreased in RBC productions have been linked to myelosuppression induced by the HAART medications and a decreased production of endogenous erythropoietin that result from neoplasm induced bone marrow infiltration (Sipsas *et al.*, 1999). Hambleton (1996) has associated the presence of other infections to be a cause of decreased RBC production in HIV infection whiles (Spivak *et al.*, 1989) have linked blunted response to erythropoietin in HIV infected individuals as a cause of decreased production of RBC. According to Coyle (1997) increased RBC destructions may be caused by auto antibodies to red blood cells and the mechanism of disseminated intravascular coagulation which involves the wide spreading of activated clotting factors resulting in blood coagulation thereby disrupting the normal functions of the blood. Haemolysis due to medications among HIV infected individuals has also been linked to increase destruction of RBC (Soriano *et al.*, 2002).

CHAPTER 3 MATERIALS AND METHODS

3.1 Study design

This was a cross sectional case control study conducted at the HIV clinic of Bomso specialist hospital in the Kumasi metropolis from August 2015 to March 2016.

3.2 Study population

One hundred and ninety two (192) confirmed HIV seropositives who consented, participated in the study. They consisted of one hundred and four (104) HIV patients already on HAART and eighty eight (88) HIV patients who were not on HAART. All participants were placed into three groups according to the center for disease control classification which indicates the CD4 lymphocytes of patients. The groups were; CD4 counts less than $200mm^{-3}$, CD4 count between 200 and $499mm^{-3}$ and the third group consisted of CD4 above $500mm^{-3}$. Ethical approval was sought from the management of Bomso hospital and the committee on human research and publication at Kwame Nkrumah University of Science and Technology (KNUST). Informed consent was also sought from all the participants.

3.2.1 Inclusion Criteria

All confirmed HIV seropositive patients who were above 18 years with no coinfection infections were recruited into the study.

3.2.2 Exclusion Criteria

Patients below 18 years, pregnant women, HIV individuals who had other confections such as tuberculosis, hepatitis B, hepatitis C and other opportunistic infections.

3.3 Anthropometric determination

Participant's weights were determined with the aid of a weighing scale to the nearest 0.1kg when they were bare footed whiles the heights were determined using a standiometer to the nearest 0.1 cm. This was used for the determination of body mass index (BMI) of the participants

3.4 Sample Collection

6ml of venous blood were taken from each patient under sterile conditions after a tourniquet has been applied for less than a minute. 3 ml out of the blood taken was placed in anticoagulated sequestrene bottles-EDTA for haematological and the immunological (CD4 and CD3) analysis. The remaining blood was centrifuged after they have been made to clot in a plain test tube. The serum obtained was stored at -20^{o}C for the assay of neopterin and some biochemical tests.

3.5 Neopterin determination

Neopterin was determined using the enzyme linked immunosorbent assay method (ELISA).

3.5.1 Test principle

The test principle applied for this test is Sandwich enzyme immunoassay. The microtiter plate provided in the kit is pre-coated with an antibody specific to neopterin. Standards and samples bind to the pre-coated antibody to form an antigen-antibody complex. This complex binds to a secondary antibody, Horseradish Peroxidase (HRP) - conjugated antibody specific to neopterin to form a stable sandwich complex. The enzyme activity of the antibody-antigen-HRP complex is obtained after washing and further binding to a substrate within a specific time frame. Sulphuric acid solution is used to terminate the enzyme-subtrate reaction. A color change is measured spectrophotometrically at a wavelength of 450nm. The concentration of neopterin is proportional to the color change that develops.

3.5.2 Procedure for neopterin determination

50µl of the standard and 10 µl of the sample were pipetted into each of the microtiter wells. It was then mixed gently for 5 seconds, covered with membrane plate and incubated for 30 minutes at 37 ℃. The content of each well were aspirated and the plates were blotted on an absorbent paper. The wells were immediately washed four times with a diluted wash buffer. To each of the wells, 50µl of the conjugate reagent was added and incubated for 30 minutes. It was then washed with diluted buffer after which 50 µl of chromogenic solutions chromogenic A and B were added to each well one after the other and incubated at 37°C for 15 minutes. The reaction was then terminated with 50 µl of stop solution to each well. The absorbance was read at 450nm using a muitiscan microplate reader within 15 minutes of the final color development.

3.6 CD4 and CD3 Count determination

CD4 and CD3 absolute T lymphocyte count was determined using the Becton Dickenson and company haematological analyzer called the BD FACS Count from California in USA.

3.6.1 Principle of CD4 determination

This involved the binding of the labeled antibodies of the fluorochrome in the reagents to the surface of the lymphocyte antigen. The sample is then run on the instrument after addition of a fixative solution onto the reagent test tubes. The fluorochrome labeled cells produces fluorescence upon the cells coming into contact with the laser light enabling the instrument to count the cells. Containing in the reagent tubes were the fluorescence reference beads which acted as a fluorescence standard for the identification of the lymphocytes and as a standard for the

quantification and calculation of the cells .The absolute counts were calculated by the automatic software by identification of the T lymphocyte populations.

3.7 Haematological assay

All the haematological parameters (Hb, WBC, RBC, HCT, MCV, MCH and MCHC) were determined using an auto analyzer known as Cell DYN1800 from Abbott Diagnostics Division (USA).

3.7.1 Haemoglobin Determination

Haemoglobin determination was based on the modified methemoglobin method. In this method the diluted sample from the camber of the white blood cells (WBC) and a lysed portion of the sample was used for the haemoglobin determination. The light emitting diodes provided the source of light by beaming through the haemoglobin cell and with the aid of a 540nm narrow band width; the cells were filtered onto the photo detector. The concentration of the haemoglobin was directly proportional to the absorbance.

3.7.2 WBC determination

Electrical impedance was used to count the White Blood Cells (WBC) by the von-Behrens white blood cell transducer as the cells passes through the aperture. A change in electrical resistance occurred as the cells passed through the aperture and generated a pulse of equivalent voltage. The number of white blood counted was equivalent to the number of pulse sensed in each cycle and the cell volume was proportional to the amplitude. In the process, WBC/RBC dilution which were in the ratio 1:25 was mixed with 1.0mL of the lysed reagent by the WBC mixing chamber.

A specified volume of the lysed sample was delivered into the counting chamber through the aperture. The WBCs were then counted by the impedance.

3.7.3 RBC Determination

This was based on the electrical impedance method which involved the mixing of 5ml of the diluent with 100ul of the sample. The RBC von-Behrens aperture then counted the cells in the sample. A specified amount of the diluted sample was then drawn into the counting camber through the aperture. The cells were then counted as RBC only if the pulse generated were above the RBCs lower threshold.

3.7.4 Mean Cell Volume (MCV)

This was computed by finding the average of the individual red blood (RBC) cell and it was measured in femtoliters (fL). MCV was derived from the size distribution data of the RBC.

3.7.5 Haematocrit (HCT)

Haematocrit is the ratio of the RBC to plasma and it is computed from the mean cell volume and the red blood cell as follows;

$$HCT = \frac{(RBC \times MCV)}{10}$$

3.7.6 Mean Cell Haemoglobin (MCH)

This is the average haemoglobin incorporated in the red blood cell. MCH is computed from RBC and Hb as follows;

$$MCH = \frac{HGB}{RBC} \times 10$$

3.7.7 Mean Cell Haemoglobin Concentration (MCHC)

This is the ratio of the haemoglobin's weight to the average volume of the red blood cell. MCHC is computed from haemoglobin and haematocrit as follows;

$$MCHC = \frac{haemoglobin}{haematocrit} \times 100$$

3.8 Biochemical assay

All the biochemical parameters including creatinine, urea, albumin, total protein, globulin, AST and ALT were assayed using an auto-analyzer known as ATAC® 8000 Random Chemistry System from USA by Elan Diagnostic System.

3.8.1 Creatinine

The Jafffe method was used in the determination of serum creatinine. In this method an orange complex colour was formed when creatinine reacted with picric acid in an alkaline medium. Creatinine in the serum was directly proportional to the colour formed and the absorbance was read at 510nm.

3.8.2 Blood Nitrogen Urea

This was based on the urease method .In this method carbon dioxide and ammonia were evolved from the hydrolyses of urea in the presence of urease and water. In the presence of NADH and

glutamate dehydrogenase, ammonia reacted with α-ketoglutarate to form L-glutamate and NAD+. The reaction proceeded with a decrease in absorbance at 340nm and this rate of change was proportional to the concentration urea in the sample.

3.8.3 Albumin

This involved the reaction of albumin with bromocresol green forming a coloured complex substance. The concentration of albumin was directly proportional to the intensity of the colour develop at 630nm.

3.8.4 Total Protein

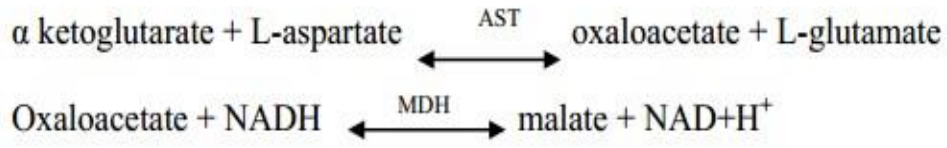
This involved the formation of a coloured complex substance as result of the reaction between protein and copper II ion. The concentration of protein in the sample was directly proportional to the intensity of the violet colour developed and the absorbance read at 540nm.

$$Protein + Cu^{2+} \xrightarrow{Alkaline} Colour\ complex$$

3.8.5 Aspartate aminotransferase

This involved the formation of oxaloacetate and L-glutamate as a result of a catalytic transfer of amino group from L-aspartate to 2 –oxoglutarate. Malate dehydrogenase catalyzes the reduction of oxaloacetate and a concurrent oxidation of NADH to NAD+. In other to prevent the interference of endogenous pyruvate usually present in the serum, lactate dehydrogenase was added to the solution. The concentration of AST in the sample was directly proportional to the decrease in absorbance at 340nm.

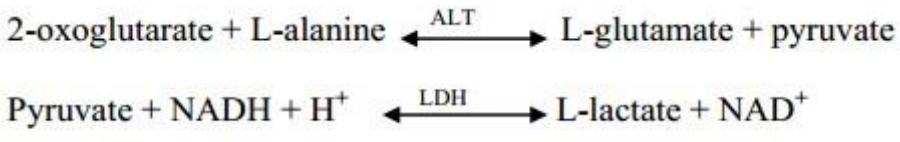
Reaction Principle



3.8.6 Alanine aminotransferase

This involved the formation of pyruvate and L-glutamate as a result of a catalytic transfer of amino group from L-alanine to 2 –oxoglutarate .Lactate dehydrogenase catalyzes the reduction of pyruvate and a concurrent oxidation of NADH to NAD+. In other to prevent the interference of endogenous pyruvate usually present in the serum, lactate dehydrogenase was added to the solution. The concentration of AST in the sample was directly proportional to the decrease in absorbance at 340nm.

Reaction Principle



3.9 Data Analysis

The data were presented as median interquartile range (IQR) for all continuous variables whiles grouped variables were expressed as proportions. Comparison between HAART naïve and HAART patients was carried out using Mann Whitney U test. Kruskal test were used where appropriate for comparison between more than two groups. Spearman correlation rank test was used to assess correlations between variables. A suitable cut off point were determined for

neopterin and all the biochemical parameters using the Youden's index which maximizes sensitivity and specificity. The performance of neopterin and the biochemical parameters were assessed using the area under the curve from the receiver operator characteristics. A probability value less than 0.05 was statistically considered to be significant. All the analysis was performed using the statistical package for social sciences version 20.

CHAPTER 4 RESULTS

4.1 Demographic and Clinical characteristics of the study population

The demographic and clinical characteristics of the studied population are shown in table 4.1A and 4.1B respectively. Out of the one hundred and ninety two (192) participants, there were more females than male for both the HAART naïve and the HAART naïve patients .The median age of the HAART group (41yrs) was statistically (p=0.203) not different from the HAART naïve group (40yrs). Out of the total participants, majority were junior high graduates for both the HAART (27.1%) and the HAART naive patients (19.8%) whiles trading (88.5%) was the major occupation of the studied participants. The median CD4 counts of the HAART group (458 mm^{-3}) was significantly (p=0.0001) higher than the HAART naïve group (229 mm^{-3}). There was a statistically significant (p=0.0001) difference between the CD3 of the HAART group (1216 mm^{-3}) and the HAART naïve group (919mm^{-3}). Although the median BMI was not statically significant (p=0.521), the HAART group had a higher BMI (23.30 kg/m^2) compared to the HAART naïve group (22.55kg/m^2). Majority of the HAART patients were on TDF+3TC+NVP (57.7%), 17.3% on CBV+NVP, 19.2 % on TDF+3TC+EFV whiles only 5.8% were on AZV+3TC+NVP. Among the studied participants, 33.9% had been diagnosed for more than four years whiles 30.7% had been on the therapy for more than four years. None of the studied participants had a history of smoking and drinking. There was also no incidence of therapy discontinuation among the HAART group.

Table 4.1A Demographic characteristics of the study population

Parameter	HAART group(104)	HAART naïve(88)	P value
Age(yrs.)	41 (35-53)	40 (31.3-50)	**0.203**
Gender	N (%)		
Male	31(16.1)	27(14.1)	
Female	73(38.0)	61(31.8)	**0.895**
Marital status			
Married	78(40.6)	63(32.8)	
Single	21(10.9)	22(11.5)	
Widowed	1(0.5)	---------	
cohabiting	4(2.1)	3(1.6)	**0.697**
Educational level			
No education	17(8.9)	14(7.3)	
Primary	20(10.4)	14(7.3)	
JHS	52(27.1)	38(19.8)	
SHS	9(4.7)	17(8.9)	
Tertiary	6(3.1)	5(2.6)	**0.311**
Occupation			
Trading	94(49.0)	76(39.6)	
Formal	5(2.6)	4(2.1)	
Students	4(2.1)	8(4.6)	
No Job	1(0.5)	--------	**0.386**

The p value for age was generated using the Mann Whitney u test whiles all the other p values were generated from the Pearson chi square. The data are presented as median interquartile range for age and the rest as proportions.

Table 4.1B Clinical characteristics of the study population

Variable	HAART group	HAART naïve	P value
CD4 (mm^{-3})	458.00(307.50-633.75)	229.00(136.25-338.75)	**0.0001**
CD3 (mm^{-3})	1216.50(931.00-1765.50)	919.00(667.50-1143.00)	**0.0001**
BMI kg/m^2)	23.30(20.33-26.85)	22.55(19.13-26.98)	0.521
HAART regime			
TDF+3TC+NVP	60(57.7)	------	
AZV+3TC+NVP	6(5.8)	------	
CBV+NVP	18(17.3)	------	
TDF+3TC+EFV	20(19.2)	------	**0.0001**
HAART duration(yrs.)		-----	
Median(IQR)	5(3-7)	-	
Group1(<2)	10(5.2)	-------	
Group2 (2-4)	35(18.2)	-------	
Group 3(>4)	59(30.7)	-------	
Duration of diagnosis(yrs.)			
Median(IQR)	5(3-8)	1(0.45-2)	
Group1(<2)	6(3.1)	53(27.6)	
Group2 (2-4)	33(17.2)	31(16.1)	
Group 3(>4)	65(33.9)	4(2.1)	**0.0001**
Smoking	------- -------	-------	
Alcohol	- ---------	------- -------	
Drug discontinuation		-	

The data are presented as median interquartile ranges for the continuous variables whiles categorical variables as proportions. The p values for CD4, CD3 and BMI were generated using the Mann Whitney u test whiles the rest were from the Pearson chi square. CD4- cluster of differentiation, IQR-interquartile range, CBV: Combivir, NVP: Nevirapine, EFV: Efavirenz, 3TC: Lamivudine, TDF: Tenofovir, AZV: Zidovudine.

4.2 HAEMATOLOGICAL PARAMETERS

4.2.1 Haemoglobin and Haematocrit

Table 4.2 shows the haematological parameters of the HAART and the HAART naïve patients. The median haemoglobin level of the HAART group (12.40g/dL) was significantly (p=0.001) higher than the HAART naïve group (11.60g/dL). There was a significant difference in haemoglobin levels between the HAART patients with CD4 counts less than 200mm^{-3} and those with CD4 count greater than 500mm^{-3} ($p \leq 0.05$). Among the HAART naïve patients, there was a statistically significant difference in haemoglobin levels when those with CD4 count less than 200mm^{-3} was compared with patients with CD4 count 200-499mm^{-3} ($p \leq 0.05$). There was a significant positive correlation between CD4 count and haemoglobin (rho 0.26, p=0.0001) and a significant negative correlation with neopterin (rho -0.26, p=0.0001) for all the studied participants (Table 4.5). Thus as the CD4 count increases there was a significant increase in haemoglobin levels but as serum neopterin increases there was a significant decrease in haemoglobin levels of the studied participants. However, there was no statistically significant correlation between haemoglobin with both CD4 and neopterin for the HAART patients but rather a significant correlation between haemoglobin with both CD4 (rho 0.29, p=0.007) and neopterin (rho -0.32, p=0.003) for the HAART naïve group (table 4.5).

The median haematocrit was significantly (p=0.001) lower in the HAART naïve group (33.45%) compared to the HAART group (35.60%).Correlation analysis of haematocrit with both CD4 count for the total participant, HAART group and HAART naïve group showed similar trend as haemoglobin.

Table 4.2 Haematological parameters of the HAART group and HAART naïve group

Parameter	HAART group	HAART naïve	P value
Hb (g/dL)	12.40 (11.60-13.28)	11.60 (10.40-12.88)	**0.001**
RBC ($\times 10^6 \mu L^{-1}$)	3.97 (3.56 -4.30)	3.96 (3.39-4.40)	0.487
WBC($\times 10^3 \mu L^{-1}$)	4.32 (3.97-5.28)	4.05 (3.20-4.792)	**0.002**
MCV(fL)	89.35 (81.05-95.65)	85.55 (78.28-92.20)	0.052
HCT (%)	35.60 (33.23-38.03)	33.45(29.78-36.78)	**0.001**
MCH (pg)	31.10 (28.68-34.48)	30.05(27.13-31.78)	**0.008**
MCHC(g/dL)	35.10 (34.13-36.10)	34.75 (33.73-35.70)	**0.032**

Data are presented as median (interquartile range) and the median comparison between HAART and HAART naïve groups by Mann Whitney U test. Hb-Haemoglobin, RBC-Red blood cell, WBC-White blood cell, MCV-Mean cell volume, HCT-Haematocrit, MCH-Mean cell haemoglobin, MCHC-Mean cell haemoglobin concentration.

4.2.2 WBC and RBC

The median WBC of the HAART group ($4.32\times 10^3 \mu L^{-1}$) was significantly (P=0.002) higher compared to that of the HAART naïve group ($4.05\times 10^3 \mu L^{-1}$). There was a significant difference in WBC levels between both the HAART and HAART group with CD4 counts less than $200mm^{-3}$ and those with CD4 count greater than $500mm^{-3}$ ($p \leq 0.05$). Among the HAART naïve patients, there was also a statistically significant difference in WBC levels when those with CD4 count less than $200mm^{-3}$ was compared with patients with CD4 count between 200-$499mm^{-3}$ ($p \leq 0.05$).

A significant positive correlation was observed between WBC with CD4 for the total participants (rho 0.41, p=0.0001), HAART group (rho 0.37, p=0.0001) and the HAART naïve group (rho 0.36, p=0.0001). Thus as the CD4 count increased there was a significant increase in WBC levels. There was however a significant negative correlations between serum neopterin with

WBC for the total participants (rho -0.41, p=0.0001), HAART group (rho -0.38, p=0.0001) and the HAART naïve group (rho -0.36, p=0.0001). This inverse relationship indicates that as serum neopterin decreases there was a significant increase in WBC counts.

Although the median RBC was statically not significant (p=0.487), the median RBC of the HAART group ($3.97\times 10^{6}\mu L^{-1}$) was higher than that of the HAART naïve group ($3.96\times 10^{6}\mu L^{1}$). Comparing the median RBC among the respective CD4 counts showed no statistical difference for the HAART group but a statistically significant difference was observed for the HAART naïve group when patients with CD4 count between 200-499 mm^{-3} was compared with those with CD4 count ≥ 500 mm^{-3} ($p\leq 0.01$), when patients with CD4 count less 200 mm^{-3} was compared with those with CD4 greater than 500 mm^{-3} ($p\leq 0.01$). A significant positive correlation was observed between RBC and CD4 count (rho 0.29, p=0.005) whiles a negative correlation was observed with neopterin (rho -0.32, p=0.002) for the HAART naïve patients (table 4.5).

Table 4.3 Comparison of the Haematological parameters of the HAART group

Parameter	CD4 COUNTS		
	<200	**200 -499**	**≥ 500**
Hb	11.75(11.35 -12.15)	12.40(11.55-13.25)	12.5(11.65-13.55)#
RBC	4.00(3.77-4.35)	4.04(3.80-4.30)	3.92(3.51-4.41)
WBC	3.93(3.36 - 4.24)	4.20(3.96-4.84)	4.92(4.08-6.86)#
MCV	85.45(76.85-88.35)	88.50(82.10-92.65)	93.10(80.85-98.40)
HCT	33.55(31.18-35.80)	35.60(33.00- 38.20)	36.20(34.10- 38.15)#
MCH	29.35(27.35-31.73)	31.00(28.50-33.30)†	33.10(29.95-35.10)#
MCHC	34.75(33.78-36.90)	35.20(34.20-36.15)	35.10(34.20-36.10)

Data are presented as median (interquartile range) and the median comparison between HAART and HAART naïve groups by Mann Whitney U test. #P≤0.05 indicates level of significance when CD4 count < 200 was compared with CD4 ≥500.

Table 4.4 Comparison of the Haematological parameters of the HAART naïve group

Parameter	CD4 COUNTS		
	<200	**200 -499**	**≥ 500**
Hb	11.00(9.73-12.50)*	11.70(10.90-12.90)	13.00(10.75-15.15)
RBC	3.74(3.02-4.30)	4.01(3.51-4.40)†	5.05(4.29-5.44)##
WBC	3.67(3.01- 4.20)*	4.19(3.62-5.18)	4.25(4.00-5.78)#
MCV	84.55(78.58-91.08)	87.80(78.60-92.60)	78.20(59.65-87.70)
HCT	32.05(28.03-35.90)*	34.60(31.70- 37.20)	36.20(31.35- 40.75)
MCH	28.65(26.80-31.50)	30.90(27.80-32.90)	27.10(20.85-32.70)
MCHC	34.15(33.05-35.28)*	34.90(34.10-35.90)	35.60(34.25-37.35)

Data are presented as median (interquartile range) and the median comparison between HAART and HAART naïve groups by Mann Whitney U test *P≤0.05 ***indicates level of significance when CD4 count < 200 was compared with CD4 200-499,*** †P≤ 0.01 ***indicate level of significance when CD4 count 200-499 was compared with CD4 count ≥ 500, #P≤ 0.05, ##P≤0.01 indicate level of significance when CD4 count < 200 was compared with CD4 ≥500.***

4.5 Correlation of Neopterin and CD4 with haematological parameters

	Total patient		**HAART patient**		**HAART naïve**	
CD4 with	Rho	P value	Rho	P value	Rho	P value
Hb	0.262	**0.0001**	0.074	0.458	0.287	**0.007**
RBC	0.096	0.187	-0.148	0.135	0.295	**0.005**
WBC	0.414	**0.0001**	0.370	**0.0001**	0.356	**0.001**
MCV	0.130	0.073	0.205	**0.037**	-0.070	0.517
HCT	0.283	**0.0001**	0.134	0.175	0.271	**0.011**
MCH	0.201	**0.005**	0.235	**0.016**	0.035	0.743
MCHC	0.131	0.070	-0.032	0.750	0.208	0.052
Neopterin with						
Hb	-0.267	**0.0001**	-0.0071	0.471	-0.316	**0.003**
RBC	-0.104	0.152	0.152	0.123	-0.323	**0.002**
WBC	-0.410	**0.0001**	-0.367	**0.0001**	-0.362	**0.001**
MCV	-0.126	0.081	-0.206	**0.036**	0.0073	0.498
HCT	-0.288	**0.0001**	-0.132	0.182	-0.298	**0.005**
MCH	-0.201	**0.005**	-0.237	**0.015**	-0.0037	0.734
MCHC	-0.140	0.053	-0.0.30	0.762	-0.227	**0.033**

4.3 BIOCHEMICAL PARAMETERS

4.3.1 Albumin, Total protein and Globulin

The biochemical parameters of the HAART and HAART naïve patients are given in table 4.7. The median serum albumin of the HAART group (40.05g/L,) was significantly (p=0.0001) higher compared to the median albumin of the HAART naïve group (34.75 g/L). A statically significant difference was observed when the albumin levels of the HAART group was compared among the respective CD4 count groups (p=0.0001). A similar trend was also observed when the albumin levels of the HAART naïve patients were compared among the respective CD4 count groups as shown in table 4.8. Albumin showed a strong positive correlation with CD4 count for the total participants (rho 0.84, p=0.0001), HAART patients (rho 0.79, p=0.0001) and the HAART naïve patients (0.69, p=0.0001). This is thus indicative that as the CD4 count increases there was a concomitant increase in the serum albumin of the studied participants. On the contrary, albumin showed a statistically significant negative correlation with serum neopterin for the total participants (rho -0.83, p=0.0001), HAART patients (rho -0.79, p=0.0001) and the HAART naïve patients (rho -0.67, p=0.0001). This inverse relationship suggests that as serum neopterin decreases there was a significant increase in the albumin levels of the studied participants.

The median total serum protein of the HAART naïve patients (118.90g/L) was significantly (p=0.0001) higher than the HAART patients (97.40g/L). Serum total protein levels were found to increase in both the HAART and the HAART naïve patients as the disease progresses (table 4.8). Total protein also showed a negative correlation with CD4 count for the total participants (rho -0.73, p=0.0001), HAART patients (rho-0.80, p=0.0001) and the HAART naïve patients (rho 0.39, p=0.0001). However a statically significant positive correlation between total protein and serum

neopterin was observed for the total participant, HAART naïve and the HAART patients (table 4.9). The median serum globulin levels of the HAART naïve patients (84.35g/L) was significantly (p=0.0001) higher than the HAART patients (57.00g/L). Correlation analysis of serum globulin with both CD4 count and serum neopterin showed similar trend to that of total protein as shown in table 4.9.

4.3.2 AST and ALT

There was a statistically significant difference in the median AST and ALT when compared between the HAART and HAART naïve patients (p=0.0001). Both AST and ALT were also found to be increased as the disease progressed. A significant negative correlation was observed between CD4 and AST for both the HAART (rho-0.59, p=0.0001) and HAART naïve patients (rho -0.51, p=0.0001). However the correlation of AST with serum neopterin was significantly positive for both the HAART (rho 0.58, p=0.0001) and HAART naïve patients (rho 0.51, p=0.0001). Correlation analysis of ALT with both CD4 count and neopterin showed similar trend to that of AST as shown in table 4.9. Negative correlations were observed between the duration of therapy treatment with both AST (rho-0.38, p=0.0001) and ALT (rho -0.35, p=0.0001). This is indicative that as the duration of therapy increases there was a concomitant decrease in the serum levels of AST and ALT as shown in the scatter plots below (figure 4.1 and 4.2 respectively).

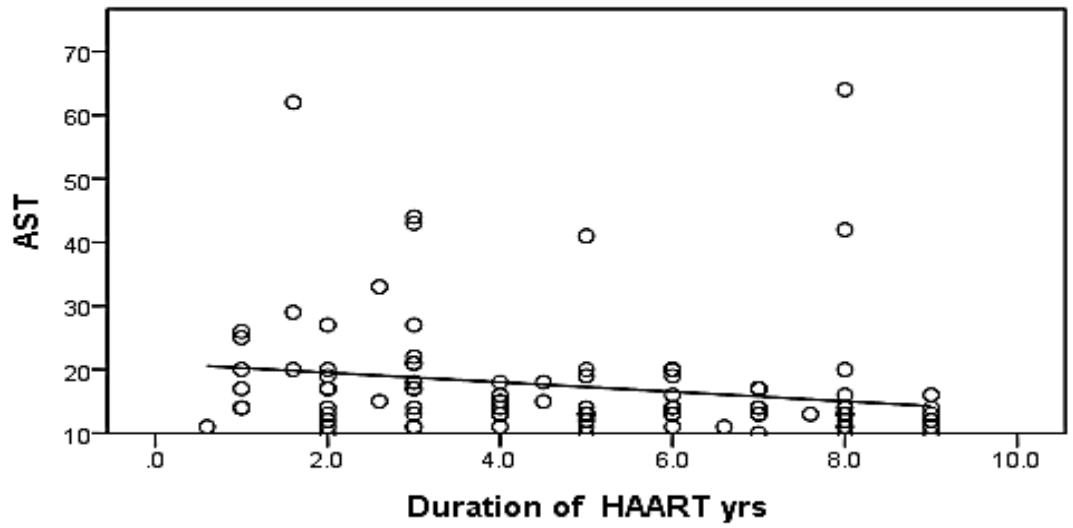


Figure 4.1 Linear relationship between AST and duration of therapy treatment

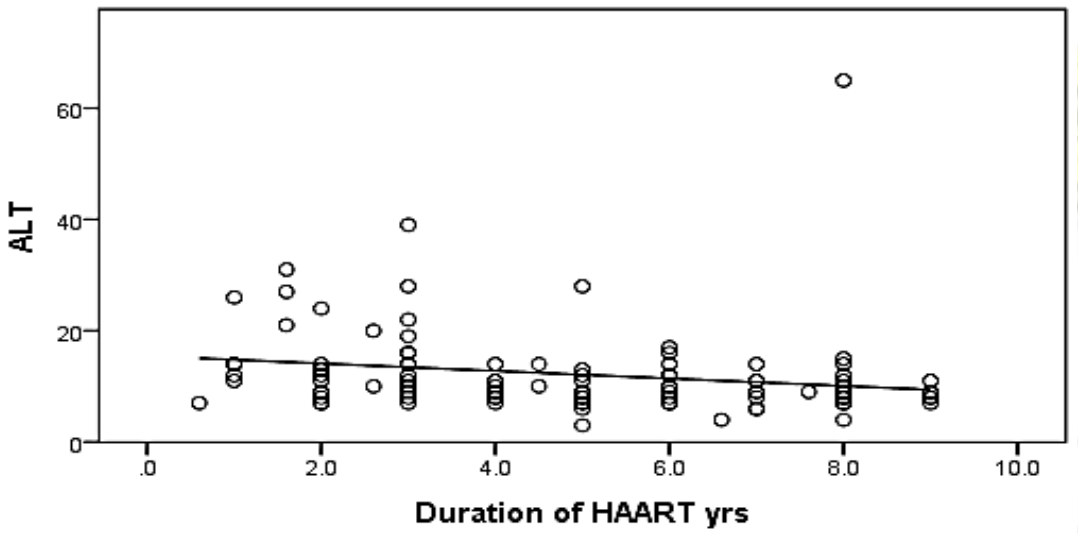


Figure 4.2 Linear relationship between ALT and duration of therapy treatment

Table 4.6 Biochemical parameters of the HAART group and HAART naïve group

Parameter	HAART group	HAART naïve	P value
Albumin (g/L)	40.05(36.50-41.28)	34.75 (32.10-39.10)	**0.0001**
Globulin (g/L)	57.00(48.95-74.98)	84.35 (69.83-92.03)	**0.0001**
Total Protein (g/L)	97.40(89.13-110.60)	118.90 (100.33-127.30)	**0.0001**
AST (U/L)	14.00(13.00-18.75)	23.00 (15.00-35.75)	**0.0001**
ALT (U/L)	10.00(8.00-14.00)	15.50 (10.00-25.00)	**0.0001**
Urea (mmol/L)	2.70(2.30-3.20)	3.20 (2.70-4.60)	**0.0001**
Creatinine(μmol/L)	62.00(55.00-69.75)	71.50 (62.00-84.50)	**0.0001**

Data are presented as median (interquartile range) and the median comparison between HAART and HAART naïve groups by Mann Whitney U test. AST-Aspartate amino Transferase, ALT-Alanine amino Transferase

Table 4.7 Comparison of Biochemical parameters of the HAART group

Parameter	CD4 COUNTS		
	<200	**200 -499**	**≥ 500**
Albumin	31.30(30.27-33.18)***	38.70(35.80-40.05)†††	41.20(40.25-42.45)###
Globulin	86.05(75.73-89.90) **	64.80(57.75-80.55)†††	48.20(42.55-52.85)###
Protein	117.20(102.43-121.53)	97.40(100.70-119.90)†††	89.10(84.35- 94.15)###
AST	19.50(16.00-29.75)	17.00(14.00-20.00)†††	13.00(11.50-14.00)###
ALT	17.50(10.25-26.50)	12.00(10.00-14.00)†††	9.00(8.00-9.00)##
Urea	2.95(2.28-5.13)	3.10(2.90-3.60)†††	2.3.00(2.20-2.50)#
Creatinine	69.00(57.00-85.25)	68.00(66.00-79.00)†††	55.00(53.00-57.50)###

*****P≤0.01, ***P≤ 0.0001 indicate level of significance when CD4 count < 200 was compared with CD4 200-499, †††P≤ 0.0001 indicate level of significance when CD4 count 200-499 was compared with CD4 count ≥ 500, #P≤0.05, ##P≤ 0.01, ###P≤ 0.0001 indicate level of significance when CD4 count < 200 was compared with CD4 ≥500***

4.8 Comparison of the biochemical parameters of the HAART naïve group

Parameter	CD4 COUNTS

	<200	200 -499	≥ 500
Albumin	32.10(30.33-33.20)***	36.40(34.30-39.30)††	40.20(39.70-40.35)###
Globulin	88.05(83.63-97.03)***	81.40(60.10-90.10)††	55.10(49.90-58.50)###
Protein	119.70(116.60-127.40)	119.90(98.30-127.20)†	95.20(89.75- 98.75)###
AST	25.50(20.50-44.00)**	18.00(14.00-30.00)†	13.00(11.00-14.5)##
ALT	21.00(12.25-28.50)**	13.00(10.00-23.00)†	8.00(7.50-10.00)##
Urea	3.60(2.65-5.10)	3.20(2.90-4.40)††	2.60(2.30-2.65)#
Creatinine	76.00(65.50-99.75)	68.00(62.00-82.00)††	55.00(53.50-60.00)##

***P≤ 0.01, ***P≤ 0.0001 indicate level of significance when CD4 count < 200 was compared with CD4 200-499, †P≤ 0.05, ††P≤ 0.01, †††P≤ 0.0001 indicate level of significance when CD4 count 200-499 was compared with CD4 count ≥ 500, #P≤ 0.05, ##P≤ 0.01, ###P≤ 0.0001 indicate level of significance when CD4 count < 200 was compared with CD4 ≥500.*

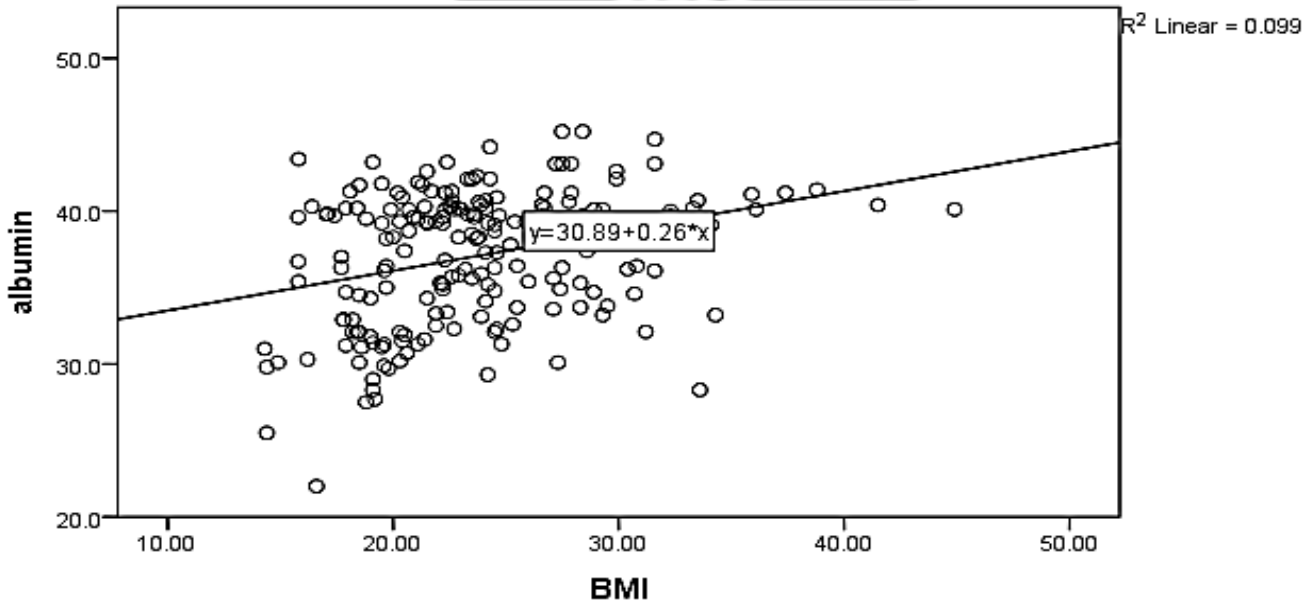


Figure 4.3 Scatter plot showing linear relationship between albumin and BMI of the studied participants.

Table 4.9 Correlation of Neopterin with CD4, biochemical parameters and some clinical factors

	Total patient		HAART patient		HAART naïve	
Neopterin with	Rho	P value	Rho	P value	Rho	P value
CD4	-0.991	0.0001	-0.995	0.0001	-0.964	0.0001
Albumin	-0.832	0.0001	-0.786	0.0001	-0.674	0.0001
Globulin	0.822	0.0001	0.866	0.0001	0.426	0.0001
Protein	0.738	0.0001	0.802	0.0001	0.580	0.0001
AST	0.628	0.0001	0.577	0.0001	0.507	0.0001
ALT	0.579	0.0001	0.506	0.0001	0.475	0.0001
Urea	0.533	0.0001	0.741	0.0001	0.124	**0.250**
Creatinine	0.584	0.0001	0.766	0.0001	0.263	0.013
BMI	-0.318	0.0001	-0.225	0.022	-0.516	0.0001
DT	-0.289	0.003	-0.289	0.003	-------	--------

	Total patient		HAART patient		HAART naïve	
CD4 with	Rho	P value	Rho	P value	Rho	P value
Albumin	0.843	0.0001	0.797	0.0001	0.696	0.0001
Globulin	-0.818	0.0001	-0.868	0.0001	-0.552	0.0001
Protein	-0.730	0.0001	-0.803	0.0001	-0.390	0.0001
AST	-0.631	0.0001	-0.592	0.0001	-0.508	0.0001
ALT	-0.584	0.0001	-0.521	0.0001	-0.478	0.0001
Urea	-0.535	0.0001	-0.749	0.0001	-0.132	**0.219**
Creatinine	-0.576	0.0001	-0.764	0.0001	-0.254	0.017
BMI	0.301	0.0001	0.218	0.026	0.462	0.0001
DT	0.297	0.002	0.297	0.002	-------	--------

Rho-Spearman correlation rank coefficient, BMI-Body mass index, DT –duration of HAART treatment

4.4 BODY MASS INDEX

The median body mass index of the HAART patients (23.30kg/m^2) was higher than the HAART naïve patients (22.55kg/m^2), however this was statistically not significant (p=0.521) .Although the median CD4 count of the HAART group did not show any significant difference when compared with the respective BMI category (p=0.139), CD4 count was found to be higher among subjects classified as obese (513mm^{-3}) and overweight (491mm^{-3}) as shown in table 4.10.

Neopterin levels were also found to be lower among the subjects classified as overweight and obese. However, CD4 count and neopterin differed significantly when compared with the

respective BMI categories of the HAART naïve patients (p=0.001). BMI showed a positive correlation with CD4 count for both the HAART patients (rho 0.22, p=0.026) and the HAART naïve patients (rho 0.46, p=0.0001).This suggests that as the CD4 increased there was a significant increase in BMI. However, BMI had a negative correlation with neopterin for both the HAART patients (rho -0.23, p=0.026) and the HAART naïve patients (rho -0.52, p=0.0001).

This is indicative that as serum neopterin decrease there was a concomitant increase in BMI.

Table 4.10 Trend analysis of BMI, CD4 and Neopterin

On HAART	**Underweight**	**Normal**	**Overweight**	**Obese**	**p value**
(N=104)	**14**	**60**	**19**	**11**	
CD4 count	409.5(172-535.5)	433(298-633.8)	491(377-820)	513(492-626)	0.139
Neopterin	29.7(22.7-63.1)	29.4(18.9-40.1)	24.5(14.1-31.9)	23.5(19.3-23.4)	0.131
HAART naïve					
(N=88)	**12**	**48**	**19**	**9**	
CD4 count	123.5(104.3-163.8)	229(136.3-375)	264(206-351)	308(219.5-450)	**0.001**
Neopterin	70.3(61.9-80.2)	52.6(32.2-68.8)	43.4(34.4-54.3)	39.1(26.9-53.2)	**0.001**

4.5 NEOPTERIN

Serum neopterin of the HAART naïve patients (51.70nmol/L) was significantly higher (p=0.0001) than the HAART patients (26.40nmol/L) as shown in the bar chart below (figure 4.4). Among the HAART patients, there was a statistically significant difference (p≤0.0001) in neopterin levels when compared with the respective CD4 count groups. HAART patients with CD4 <200mm^{-3} had the highest median serum neopterin value (70.10nmol/L) whiles the group

-3 with CD4 ≥500mm had the lowest median serum neopterin value (17.20nmol/L). A strong negative correlation was observed between serum neopterin and CD4 counts for both the HAART (rho -0.99, p=0.0001) and the HAART naïve patients (rho-0.96, p=0.0001). This is thus indicative that as the CD4 count decreased there was a significant increase in the serum neopterin of the studied participants. Correlation analysis of neopterin with the biochemical parameters was all statistically significant except urea (table 4.9). Similar trend was also observed for correlation analysis of CD4 count with the biochemical parameters as shown in table 4.9. There was also a statistically significant difference (p≤0.0001) in the serum neopterin levels of the HAART naïve patients when compared among the respective CD4 count groups as shown in the box plot below (figure 4.6). Serum neopterin were found to be increased as the disease progresses in both the HAART and the HAART naïve patients (figure 4.5 and 4.6 respectively). Neopterin levels were also found to be decreased as the duration of the therapy increases with patients being on the therapy for more than four having the lowest serum neopterin levels followed by those who have been on the therapy between two and four years. Highest neopterin values were found in patients who have been on the therapy for less than two years as shown in the bar chart below (figure 4.7)

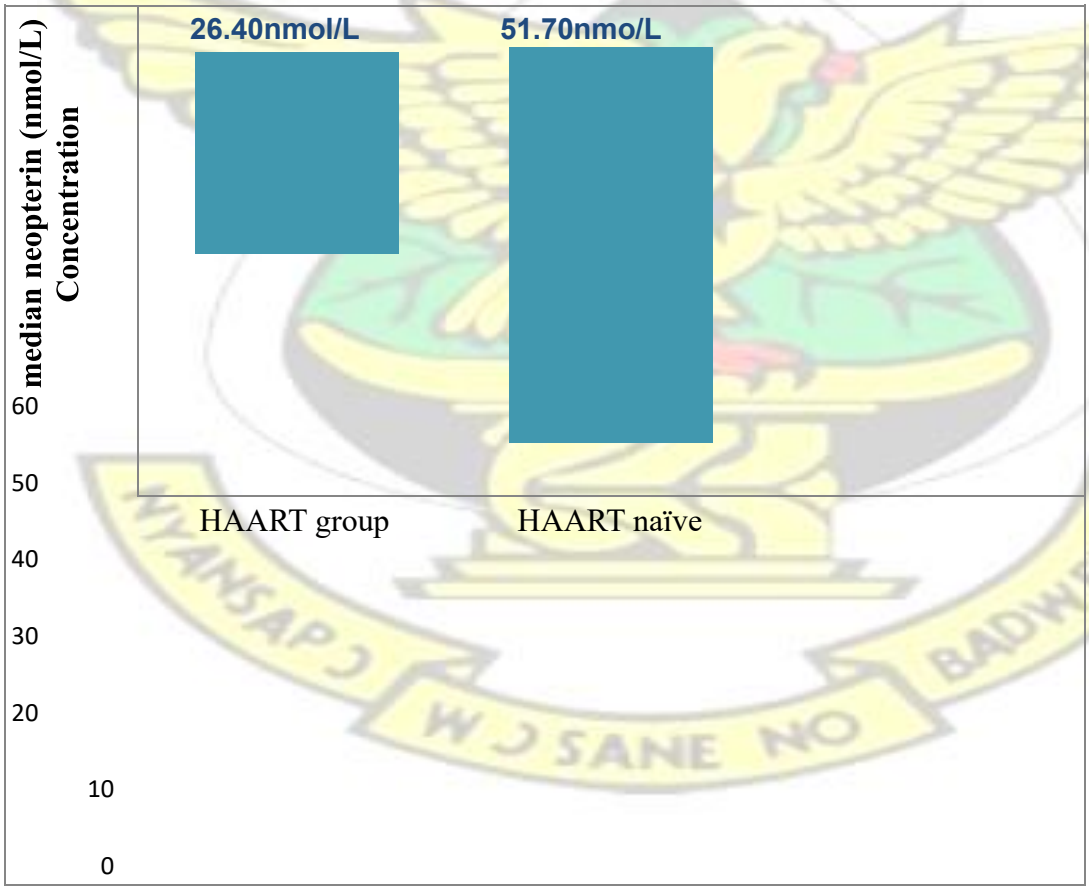


Figure 4.4 Comparison of serum neopterin levels in the HAART and the HAART naïve patients. (P value=0.0001).

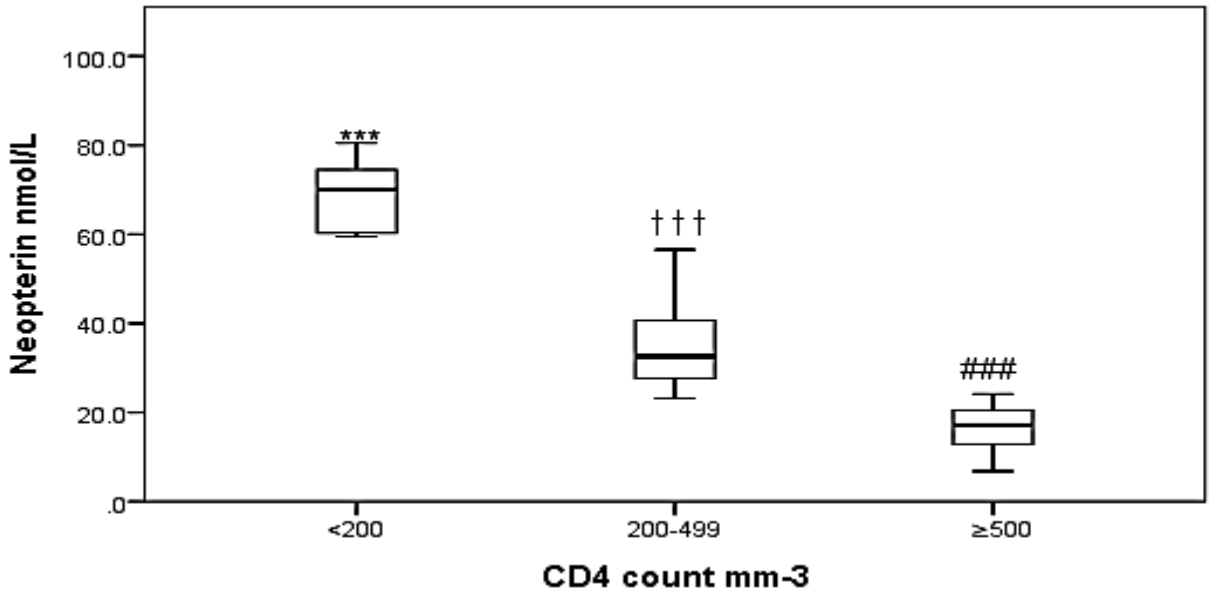


*Figure 4.5 Comparison of neopterin concentration in HAART patients with the CD4 categories.***P≤ 0.0001, †††P≤ 0.0001 , ###P≤ 0.0001 indicate level of significance when CD4 count < 200 was compared with CD4 200-499, CD4 count 200-499 with CD4 count ≥ 500 and CD4 count < 200 compared with CD4 ≥500 respectively.*

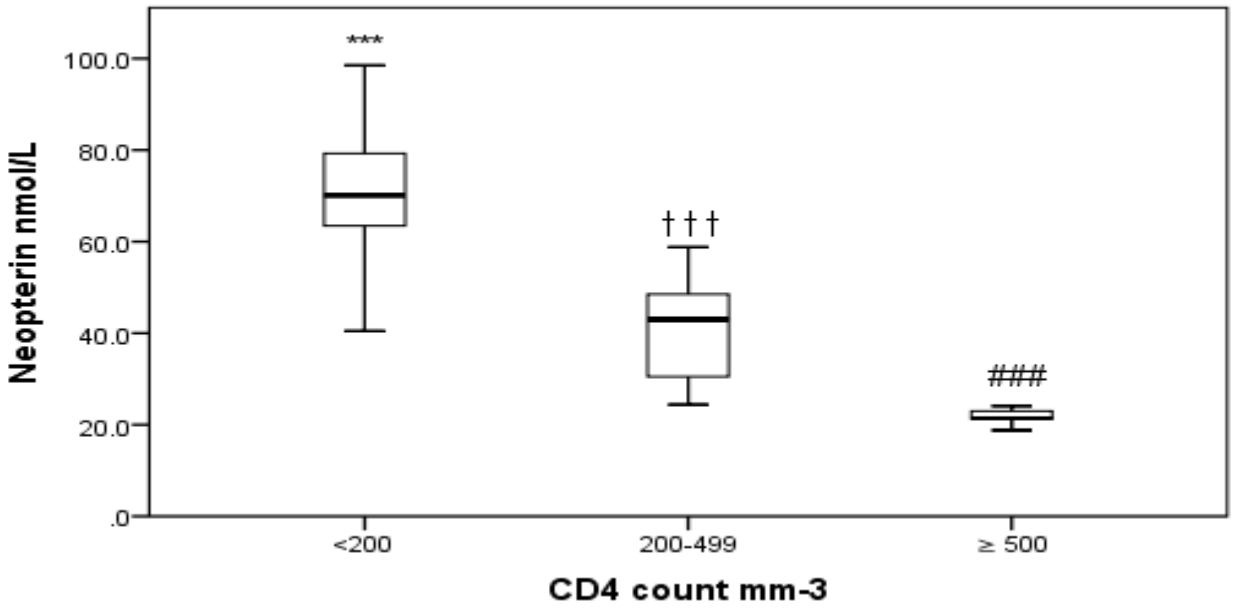


*Figure 4.6 Comparison of neopterin concentration in HAART naïve patients with the CD4 categories.***P≤ 0.0001, †††P≤ 0.0001 , ###P≤ 0.0001 indicate level of significance when*

CD4 count < 200 was compared with CD4 200-499, CD4 count 200-499 with CD4 count ≥ 500 and CD4 count < 200 compared with CD4 ≥500 respectively.

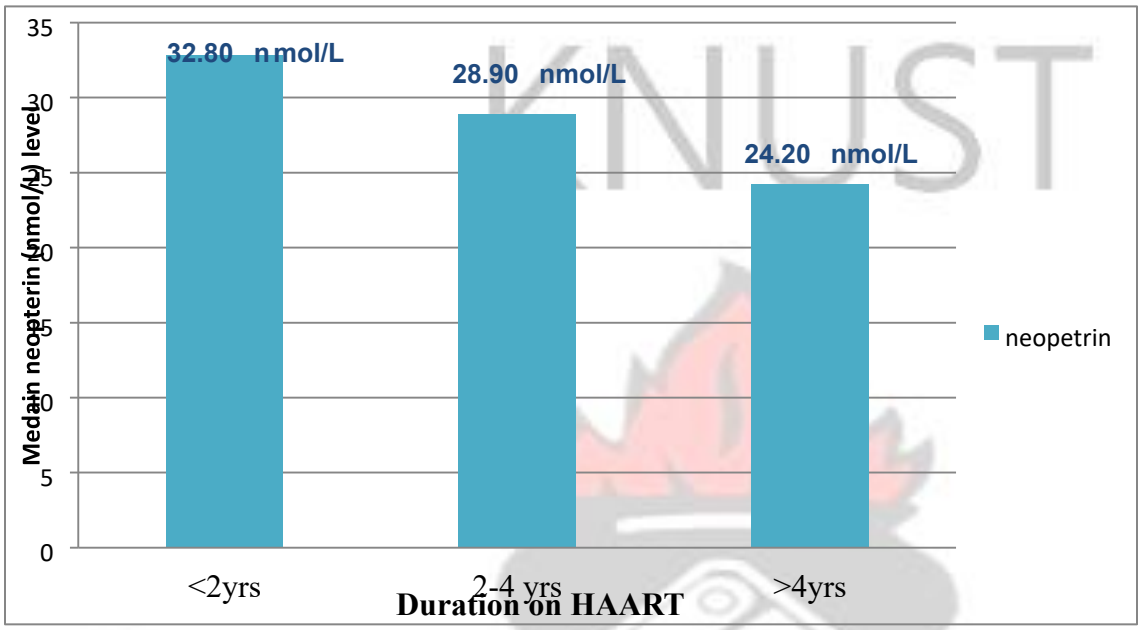


Figure 4.7 Duration on HAART in years and serum neopterin levels among the HAART patients.

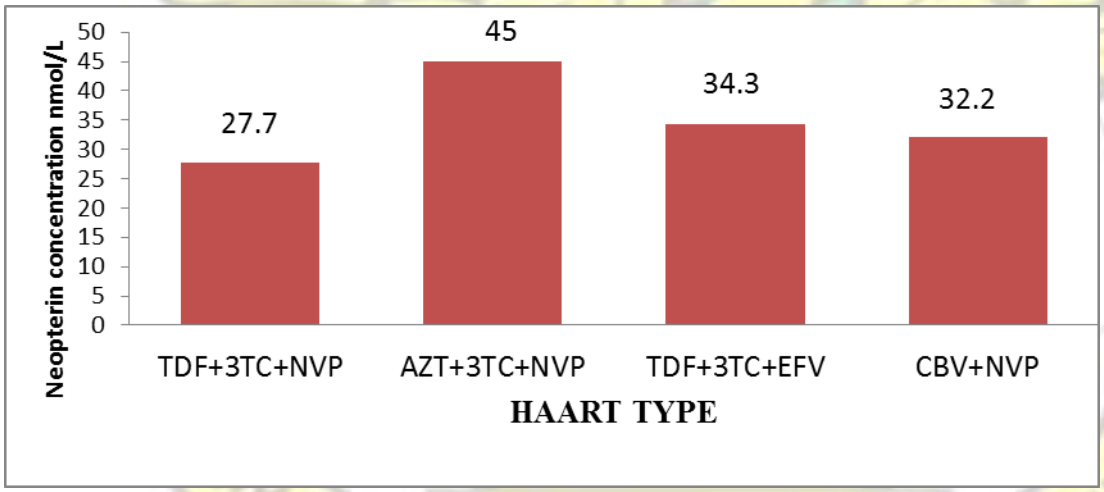


Figure 4.8 HAART types and their mean serum neopterin levels

Among the HAART group, patients on Tenofovir, Lamivudine and Nevirapine combination had the lowest mean serum levels 27.7nmol/L whiles patients on Zidovudine, Lamivudine and Nevirapine combination had the highest mea serum neopterin levels of 45.nmol/L (Figure 4.8).

Par	Neop	TP	Glb	Alb	AST	ALT	Urea	Crt
AUC	0.99	0.79	0.85	0.092	0.77	0.76	0.61	0.68
SDE	0.006	0.032	0.027	0.025	0.04	0.42	0.052	0.047
95% CI	0.98-1.0	0.72-0.85	0.80-0.91	0.04-0.14	0.69-0.85	0.68-0.85	0.51-0.71	0.59-0.77
p value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.021	<0.0001
Cut-off	55.4nmol/L	102.5g/l	72.65g/l		20.5U/L	16.5U/L	3.65	69.5
Sens	97.5%	95.7%	95.7%		69.9%	65.2%	45.7%	65.2%
Spec	95.9%	64.4%	67.8%		80.1%	84.2%	80.8%	71.9%

Table 4.11 Receiver Operator Characteristics of the Biochemical Parameters

Par: parameter SDE: Standard deviation error, AUC: area under the curve, CI: confidence interval, Sen: sensitivity, Spec: specificity, Neop: neopterin, TP: total protein, Glb: Globulin, Alb: albumin, Crt: creatinine, AST-Aspartate amino transferase, ALT-Alanine amino transferase.

Table 4.12 Diagnostic accuracy of serum neopterin in predicting CD4 counts < 200mm^{-3}

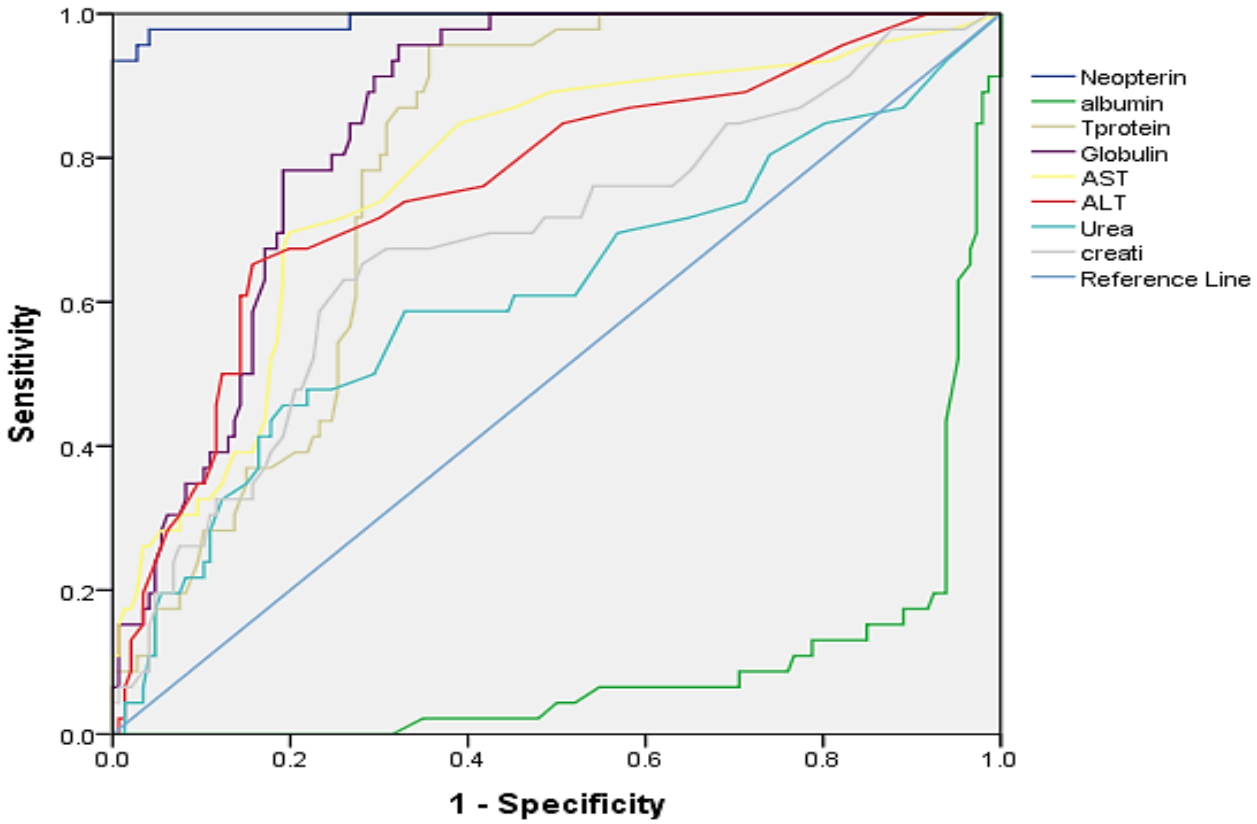
	Cut off	Sen	Spec	PPV	NPV	TP	TN	FP	FN	Accu	AUC
Total subjects	55.4nmol/L	97.5%	95.9%	0.913	0.815	42	119	4	27	0.839	0.99
HAART group	58.0nmol/L	100%	100%	0.90	0.904	9	85	1	9	0.904	1.00
HAART naïve	59.5nmol/L	91.7%	100%	0.806	0.942	29	49	7	3	0.886	0.98

Sen; sensitivity, Spec; specificity, Accu; Accuracy, PPV; Positive predictive value, NPV; Negative predictive value, TP; True positive, TN; True negative, FP; False positive, FN; False negative, AUC; Area under the curve.

4.6 Receiver operator characteristics

The receiver operator characteristics were performed for all the biochemical parameters to asses which parameter could best predict the HIV disease progression as shown in table 4.7 above. Neopterin was the best marker as seen in the area under the curve (0.99). From the Youden's index J (specificity+sensitivity-1) neopterin gave the highest sensitivity (97.5%) and specificity

(95.9%) at the cut off of 55.4nmol/L for the total participants. From table 4.12, the diagnostic accuracy of serum neopterin in predicting CD4 less than 200mm^{-3} was found to be 83.9% for the total participants. The area under the curve were also 0.99,1.0 and 0.98 for the total subject,



HAART and HAART naïve patients repectively.

Figure 4.9 ROC curves of neopterin and the biochemical parameters

CHAPTER 5 DISCUSSION

Several studies have shown the value of prognostic markers such as CD4 counts, viral RNA loads and soluble markers of immune activation in predicting the disease progression but the principal biomarker marker used in the monitoring of HIV infected individuals is the CD4 count. However, CD4 counts estimation is expensive and require considerable skills hence few laboratories in resource limited settings offer the test for patients making the clinical monitoring of patients difficult. Considering the fact that HIV infection is mostly endemic in poor developing/third world countries where resources and infrastructures are limited, there is the need for cost effective, easily performed and readily available surrogate markers that can assist in predicting the disease progression and patient's response to HAART.

5.1 HAEMATOLOGICAL PARAMETERS IN HIV DISEASE PROGRESSION

Haemoglobin was significantly (p=0.001) higher in the HAART group (12.40g/dL) than the HAART naïve patients (11.60g/dL).This agrees with earlier findings by (Enawgaw *et al.*, 2014; Bipath *et al.*, 2015).Haematocrit also showed a significant increase in the HAART group compared to the HAART naïve group. Consistent with these findings, Odunukwe *et al.* (2005) and Belperio and Rhew (2004) also showed improvement in haemoglobin and haematocrit levels in their study but inconsistent with an earlier report by (Omoregie *et al.*, 2008) where they reported no significant difference in haematocrit values between HAART and HAART naïve patients. They however attributed their findings to the usage of Zidovudine as one of the HAART agents (monotherapy) which was not the case in this study where all the HAART patients used combined therapies. Although Mildvan (2003) also reported that HAART patients still develop anaemia, he did attribute it to the presence of hepatitis C infection among HAART patients who were given other drugs

such ribavirin. Because this present study excluded patients with hepatitis C infection and the HAART patients were not on any other drug, it is still indicative that the usage of HAART is beneficial in promoting the synthesis of blood cells among HIV seropositive on HAART which also confirms a recent findings by (Owiredu *et al.*, 2011). Abrams *et al.* (2000) reported that small increase in haemoglobin level (up to 2 g dL-1) were associated with a beneficial effect on total quality of life. Comparing haemoglobin levels with the respective CD4 count categories found haemoglobin levels to be decreased as the disease progresses in the studied participants with patients whose CD4 was below $200mm^{-3}$ having the lowest haemoglobin levels. This is consistent with the findings of (Anastos *et al.*, 2004; Costello *et al.*, 2005). Hence anaemia may correlate with the severity of the HIV infection especially in patients with CD4 count less than $200mm^{-3}$. Earlier reports have demonstrated that low levels of haemoglobin may be associated with AIDS and death in people with AIDS (Saah *et al.*, 1994; Obirikorang and Yeboah, 2009) indicating the prognostic value of haemoglobin measurement in the disease progression as evident in this study. Decreased haemoglobin in HIV patients could be due to several factors including insufficient dietary intake, bleeding and bone marrow suppression which are caused by the infection of the progenitor cells, therapeutic treatment of HIV related malignancies. Furthermore, cytokines involvement in inhibiting hematopoietic cells in the bone marrow could also lead to decreased haemoglobin levels in HIV infected individuals. The significant positive correlation observed between haemoglobin levels and CD4 count in the studied participants coupled with the decrease in haemoglobin levels as the disease progresses are indicative that haemoglobin measurement may independently provide prognostic information of that provided by CD4 counts. The incidence of leucopenia in HIV infected patients has been shown to correlate with the severity of the disease progression (Opie, 2012; Moolla *et al.*, 2015) which is consistent with the findings of this study which found WBC to be decreased as the disease progresses. The significant negative correlation observed between

WBC and serum neopterin levels in both the HAART (rho-0.37, p=0.0001) and HAART naïve patients (rho-0.36, p=0.0001) clearly shows that immune activation which result in the production several soluble markers may be increased as a results of the reduction in number of monocyte/macrophages which functions as antigen presenting cells. In the pathogenesis of HIV infection, the pro inflammatory released may accelerate the death of activated cells and suppress the regeneration of immune cells in the bone marrow, lymph node and the thymus which may contribute to the decline in WBC among the HAART naïve patients (Opie, 2012).

5.2 BIOCHEMICAL PARAMETERS IN HIV DISEASE PROGRESSION

Serum albumin levels in the highly active antiretroviral patients was appreciably higher than the naïve highly active antiretroviral patients (p=0.0001). Comparing the albumin levels with the respective CD4 counts showed a significant and a positive correlation for both the HAART and HAART naïve group. Hence as the disease progressed there was a concomitant decrease in the albumin levels. These findings tie with earlier reports by (Olawumi and Olatunji, 2006). In a community based cohort study, Mehta *et al.* (2006) observed an albumin level of <35g/l to be associated with a faster progression to AIDS after measuring albumin concentration before and after the presence of detectable HIV antibodies. Low albumin levels in the HAART naïve group may be explained by poor nutritional status and this was supported by the positive correlation observed between BMI and serum albumin for the studied participants (figure 4.3) since BMI is an indicator of one's nutritional status (Langford *et al.*, 2007). Other possible mechanisms for decreased albumin may be due to the persistent inflammatory response caused by the infection.

Also since the concentrations of acute phase proteins are decreased in chronic inflammation as a result of elevation in cytokines levels which imposes the liver to channel other proteins needed for immune response, it was not surprise that albumin levels were decreased in the HAART naïve

patients given the fact that albumin is a negative acute phase protein. This and other previous reports buttress the premise that albumin measurement in HIV infected persons may provide information on the prognosis and the progression of the HIV disease (Feldman *et al.*, 2003; Bipath *et al.*, 2015). There was a significantly higher total protein in the naïve highly active antiretroviral patients than the highly active antiretroviral patients (p=0.0001). This result tie with earlier report by Abubakar *et al.* (2015) .Several mechanism have been put forward regarding derangements in serum total protein levels during the infection. During HIV infection there is an acute phase response to the infection leading to the high protein turn over and the degradation of serum proteins (Abubakar *et al.*, 2015). Fleck (1989) reported that HIV infection induces proliferation of leucocyte, increase in immunoglobulins and cytokine synthesis which contributes to protein turn over. High serum protein during HIV infection could also be attributed to tissue breakdown and degeneration (Stahl *et al.*, 1982) . The serum globulin levels of the HAART naïve patients were significantly higher than the HAART experienced patients. Consistent with this finding, other previous cross sectional studies that have evaluated serum globulins have found reduction in serum globulin in HAART patients compared to HAART naïve patients (Jacobson *et al.*, 2002; Serpa *et al.*, 2010). This observation could be attributed to the chronic immune activation and B cell dysfunction which induces hypergamaglobulinemia via polyclonal B cell activation leading to a spontaneous increase in immunoglobulins which may result in the elevation of serum globulin in the HAART naïve patients (Moir and Fauci, 2009).

Liver enzyme elevation during antiretroviral therapy has been documented as one of the major causes of mortality and morbidity (Ejilemele *et al.*, 2007). The median AST and ALT of the naïve highly active antiretroviral patients were higher than the highly active antiretroviral patients. This concur with earlier findings by Osakunor *et al.* (2015b) and inconsistent with Lucien *et al.* (2010) who reported increased AST and ALT levels only after the initiation of

HAART .A cross sectional case control study by Ngala *et al.* (2015) also found an increased AST and ALT levels among HAART experienced patients compared to HAART naïve patients which contradict the findings of this present study. The difference could be attributed to the fact in their study about 9% of HAART patients were coinfected with hepatitis B but was not the case in this study which excluded all patients infected with hepatitis B. Other studies on hepatotoxicity have shown the existence of hepatic damage during HAART in the presence of other coinfection such as tuberculosis (Heil *et al.*, 2010). Since patients with confections where excluded from this study, the raised ALT and AST levels in the HAART naïve group could be attributed to the HIV infection itself as a result of chronic immune dysfunction associated with raised inflammatory cytokines. These findings buttress the point that HIV patients have the tendency to develop liver enzyme elevation even in the absence of HAART (Ayelagbe *et al.*, 2014). Correlation analysis of the transaminases (AST and ALT) with the duration of therapy treatment was significantly negative ($p=0.0001$). This result is consistent with the findings of (French *et al.*, 2004) where they confirmed decreased concentration of transaminases as the treatment duration increases and inconsistent with reports by (Lucien *et al.*, 2010; Osakunor *et al.*, 2015a).Clearly the course of HIV results in a number of biochemical derangements which correlate closely with the disease progression and an indicator of treatment response to HAART.

5.3 BODY MASS INDEX AND HIV DISEASE PROGRESSION

Body mass index has been shown to be of importance in the disease management due to the presence of wasting syndrome which occur during the HIV infection (Palella Jr *et al.*, 1998). There was no statistical difference in the body mass index of the HAART and HAART naïve patients ($p=0.502$). BMI was found to correlate positively with CD4 count for both the HAART and HAART naïve patients. Concurrent with this findings, previous studies have reported higher BMI to be associated with increased CD4 counts as well as improved survival resulting in a slower

disease progression (Jones *et al.*, 2003; Denue *et al.*, 2013) .Earlier report have also found a higher BMI to correlate with an improved immune reconstitution and slower progression to AIDS among HAART patients (Koethe *et al.*, 2011). The strong positive correlation between BMI and CD4 count is indicative that increased BMI may be associated with immunological improvement and a reduction in immune activation which may results in to a decrease in serum neopterin levels. In support of this, the study found serum neopterin to correlate negatively with CD4 count for both the HAART and the HAART naïve patients. In the pre-HAART era, being underweight was associated with lower CD4 cell counts and shorter survival, while higher weight was associated with slower disease progression (Guenter *et al.*, 1993; Palenicek *et al.*, 1995; Wheeler *et al.*, 1998; Shor-Posner *et al.*, 2000) which also confirms the results of this study which found a higher BMI to correlate closely with an increased CD4 count in both the HAART and HAART naïve patients (table 5).Hence, although being overweight and obese has been suggested to be associated with several metabolic syndromes, this study confirms previous findings that excess adiposity may culminate in the reduction of HIV disease progression and HIV related deaths (Shuter *et al.*, 2001; Calle *et al.*, 2003) through improvement in immune reconstitution. Given it strong relationship with CD4 counts and serum neopterin, it could be measured frequently in resource limited settings. Regular measurement of body mass index may provide clinicians with some prognostic information on the disease progression enhancing close monitoring and therapeutic interventions for individuals with a greater possibility of progressing with the disease.

5.4 NEOPTERIN IN HIV DISEASE PROGRESSION

Serum neopterin levels were significantly lower in patients who were on HAART the than the HAART patients (p=0.0001). This result correlates well with studies by Amirayan-Chevillard *et al.* (2000) who showed that HAART significantly decrease circulating levels of neopterin by 30 %

(61.7nmol/L and 88.1nmol/L for HAART and HAART naïve respectively). HIV infection is associated with a continuous immune activation which stimulates the release of inflammatory cytokines thereby increasing the total level of neopterin in the HAART naïve group. Several explanations could be attributed to the increased immune activation seen in HIV infection. At the site of HIV infection, there is migration of tissue peripheral CD4 cells which results in the activation of macrophages. This leads to a reduction in the peripheral blood CD4 cells thereby contributing to a persistent immune activation. In support of this mechanism, the study found CD4 count to correlate negatively with serum neopterin for both the HAART and the HAART naïve patients suggesting that a decrease in CD4 count may result in an increase in serum neopterin. Neopterin levels were found to be increased as the disease progresses and this collaborate with studies by Chadha *et al.* (2013) who reported higher neopterin values in patients with CD4 counts less than $200mm^{-3}$. In a recent cross sectional study aimed at assessing the potential of neopterin as marker of HIV-1 disease progression, Bipath *et al.* (2015) found a higher serum neopterin in HAART naïve patients compared to HAART patients which is consistent with the findings of this study. Mildvan *et al.* (2005a) also reported that elevated baseline neopterin levels are linked with increased risk of HIV disease progression and indicate the predictive value of neopterin in the disease progression. Several explanations have been put forward as to the mechanisms of HAART on serum neopterin levels among HIV infected individuals. HAART increase $CD4^+$ T cell count (Collier *et al.*, 1996), enhances a partial recovery of immune functions and restores progenitor cell functions (Nielsen *et al.*, 1998). This give rise to changes in the subset of the T cells (Pakker *et al.*, 1998) and the restoration of interleukin 2 production (IL 2) which modulate the various aspect of the immune response (Daniel *et al.*, 1999). These earlier assertions have been buttressed by a more recent study indicating that HAART down modulate the production of cytokine which are involved

in the pathogenesis of HIV infection resulting in the reduction of circulating levels of neopterin (Wirleitner *et al.*, 2005) which confirms the findings of this present study. The significant negative correlation observed between serum neopterin and the duration of therapy treatment supports previous report that prolonged treatment with HAART reduces monocyte activation and normalises circulating neopterin levels significantly (Amirayan-Chevillard *et al.*, 2000).Although HAART was associated with a decrease in serum neopterin levels, the usage of Tenofovir, Lamivudine and Nevirapine combination resulted in a much more decrease in serum neopterin levels compared with the other HAART combinations used in this study.This clearly shows that neopterin values may be prognostic in the disease progression and an indicator of patient's treatment response to HAART.

5.5 RECEIVER OPERATOR CHARACTERISTICS

Using the area under the curve and the diagnostic accuracy from the receiver operator characteristics analysis, neopterin was the best predictor of the disease progression. At cut-off point of 55.4 nmol/L neopterin had a sensitivity and specificity of 97.5% and 95.9% respectively.

This observation suggests that neopterin could be utilized in the initiation of HAART for HIV infected patients and the monitoring of patients response to the therapy. In HIV individuals with CD4 counts less 200 mm^{-3} serum neopterin is helpful in predicting the disease progression.

CHAPTER 6

6.0 CONCLUSIONS AND RECOMMENDATIONS

This study has proved the impact of HAART in reducing AIDS related illness through improvement in haemoglobin and haematocrit levels. Hence considering the fact that they are relatively cheaper and easier to perform than CD4 and viral assay, their measurement may be useful in the monitoring of patients in resource limited settings.

It is clear from this study that HAART may have a minimal effect on hepatic enzymes among the studied population and longer duration of treatment may not result in liver enzyme elevation.

The result of this study provide evidence that increased BMI may be associated with improved immune reconstitution and suggest that measurement of BMI could be of importance in the monitoring of treatment response to HAART especially in resource limited settings.

The study has showed a lower serum neopterin levels among HAART patients compared to HAART naïve patients indicating the benefit of HAART in reducing serum neopterin levels significantly. However, the usage of Tenofovir, Lamivudine and Nevirapine combination resulted in a much more decrease in serum neopterin levels compared with the other HAART combinations used in this study. This study also found a significant negative correlation between serum neopterin and CD4 counts. Given it strong negative correlation with CD4 counts, regular measurement of serum neopterin may provide clinicians with some prognostic information on the disease progression enhancing close monitoring, host response to the therapy and therapeutic interventions for individuals with a greater possibility of progressing with the disease. With an area under the curve of 0.99, sensitivity of 97.5% and a specificity of 95.9% it may add some prognostic information to the CD4 counts used in the monitoring of the HIV disease progression. With positive predictive value of 84% and negative predictive value of 91%, serum neopterin is useful in predicting the disease progression/prognosis in patients with CD4 less than 200mm^{-3}.

Based on the findings of this study, it is recommended that larger studies on the effect of HAART on serum neopterin should be carried out to ascertain the levels of serum neopterin that suggest an appropriate treatment response and predicts the disease progression. This can complement the CD4 counts used for the monitoring of HIV infected individuals thereby enhancing the management of HIV disease in Ghana.

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APPENDIX 1

QUESTIONNAIRE

Kwame Nkrumah University of Science and Technology College of Health Science School Of Medical Science Department of Molecular Medicine

I am a postgraduate student of the institution named above doing a project titled **"Neopterin as a surrogate marker to monitor HIV disease progression and patient's response to antiretroviral therapy.** It will be appreciated if you could assist me by answering the following questions. I assure you that all information provided shall be treated confidentially.

Code…… Date…….

A. BACKGROUND AND SOCIODEMOGRAPHIC CHARACTERISTICS

Age…… Gender: M [ ] F [ ]

Marital status: Married [ ] Single [ ] Divorced [ ] Widowed [ ] Cohabiting [ ]

Occupation…….

Monthly income (GHC) ……

Religion: Christian [ ] Muslim [ ] Traditional [ ]

Ethnicity…….

Educational level: No education [ ] Primary [ ] Junior [ ] Secondary [ ] Tertiary [ ]

B. ANTHROPOMETRY

Height…….. (m) Weight ……. (Kg) BMI……. (Kg/m^2)

C. HEALTH FACTORS/MEDICATION AND LIFE STYLE

Duration of HIV infection…….months/years

Are you on HAART? Yes [ ] No [ ]

The type of HAART being taken: Combivir + Nevirapine [ ] Zidovudine + Lamivudine Nevirapine [ ] Tenofovir +Nevirapine+Lamivudine [ ] Tenofovir + Lamivudine +Efavirenz
Are you on another medication apart from HAART? Yes [ ] No [ ]

Have you ever discontinued the therapy before? Yes [ ] No [ ]

I f yes to the above for how long did you discontinued the therapy……

Do you smoke? Yes [ ] No [ ]

Do you drink alcohol? Yes [ ] No [ ]

D. LABORATORY INVESTIGATIONS

Haematological parameters

Hb…….. (g/dL)

RBC…….($\times 10^6 \mu$/L)

WBC…….($\times 10^3 \mu$/L)

MCV……. (fL)

HCT……. (%)

MCH…… (pg)

MCHC……. (gd/L)

Biochemical parameters

Albumin…….. (g/L)

Total protein…… (g/L)

Globulin…… (g/L)

AST……. (U/L)

ALT…….. (U/L)

Creatinine……. (μmol/L)

Urea…… (mmol/L)

Immunological parameters

CD4……. (mm^{-3})

CD3…… (mm^{-3})

Neopterin…… (nmol/L)

APPENDIX 2

Participant Information Leaflet and Consent Form

Kwame Nkrumah University of Science and Technology College of Health Science School Of Medical Science Department of Molecular Medicine

Title of Research:
NEOPTERIN AS A SURROGATE MARKER TO MONITOR HIV DISEASE PROGRESSION AND PATIENTS RESPONSE TO ANTIRETROVIRAL THERAPY

Name(s) and affiliation(s) of researcher(s):
This study is being conducted by Dr Christian Obirikorang, Kwantwi Boafo Louis and Prof. Margaret Frempong all of the Department of Molecular Medicine, School of Medical Sciences, KNUST

Background (Please explain simply and briefly what the study is about):
Neopterin has shown to be a well-established and a reliable marker in HIV-1 infection. Serum neopterin will be measured in the samples of HIV patients. Also hematological and biochemical assay will be measured. This will be used to determine patient response to the antiretroviral therapy and the progression of the disease.

Purpose(s) of research: The purpose of this study is to determine patient's response to antiretroviral drugs and to monitor the disease progression by measuring serum neopterin and other

biochemical and haematological parameters in HIV seropositive on HAART and HAART naïve HIV patients.

Procedure of the research, what shall be required of each participant and approximate total number of participants that would be involved in the research:
A total of 192 participants will be recruited into this study on their own accord and without any coercion. Consented Subjects will be required to sign a consent form and answer few questions in a questionnaire. Also blood samples will be taken from patients for neopterin measurements and assay of the various biochemical and haematological tests.

Risk(s):
During the study participants will be asked to disclose information they may consider private. Possible inconvenience or discomfort may result during blood sample collection

Benefit(s):
Measuring serum neopterin in HIV HAART naive patients and HIV patients on HAART will be useful in monitoring HIV disease progression and HIV disease management and the efficacy of the therapy in HAART era. This will also pave the way to better understand the immune pathogenesis of HIV disease and improve laboratory testing strategies that can be used in predicting clinical stage, initiation of HAART and management of HIV disease in Ghana.

Confidentiality: All information collected in this study will be given code numbers. No name will be recorded. Data collected cannot be linked to you in anyway. No name or identifier will be used in any publication or reports from this study.

Voluntariness: This research is entirely voluntary. You should not feel compelled to participate under any circumstances**.**

Alternatives to participation:
If you choose not to participate, this will not affect your treatment in this hospital in a way.

Withdrawal from the research:
You may choose to withdraw from the research at any time without having to explain yourself. You may also choose not to answer any question you find uncomfortable or private.

Consequence of Withdrawal:
There will be no consequence, loss of benefit or care to you if you choose to withdraw from the study.
Costs/Compensation:
Transport to the hospital will be paid by the principal investigator.

Contacts:

If you have any question concerning this study, please do not hesitate to contact Dr Christian Obirikorang, Kwantwi Boafo Louis and Prof Margaret Frempong all of Molecular Medicine Department, School of Medical Sciences, Kumasi

Further, if you have any concern about the conduct of this study, your welfare or your rights as a research participant, you may contact:

The Office of the Chairman Committee on Human Research and Publication Ethics Kumasi Tel: 03220 63248 or 020 5453785

APPENDIX 3

CONSENT FORM

Statement of person obtaining informed consent:

I have fully explained this research to _____ and have given sufficient information about the study, including that on procedures, risks and benefits, to enable the prospective participant make an informed decision to or not to participate.

DATE: _____ NAME: _____

Statement of person giving consent:

I have read the information on this study/research or have had it translated into a language I understand. I have also talked it over with the interviewer to my satisfaction.

I understand that my participation is voluntary (not compulsory).

I know enough about the purpose, methods, risks and benefits of the research study to decide that I want to take part in it.

I understand that I may freely stop being part of this study at any time without having to explain myself.

I have received a copy of this information leaflet and consent form to keep for myself.

NAME: _____

DATE: _____ SIGNATURE/THUMB PRINT: _____

Statement of person witnessing consent (Process for Non-Literate Participants):

I _____ (Name of Witness) certify that information given to (Name of Participant), in the local language, is a true reflection of what l have read from the study Participant Information Leaflet, attached.

WITNESS' SIGNATURE (maintain if participant is non-literate): _____