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# PROTEIN PROFILE OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) PATIENTS ON ANTIRETROVIRAL DRUGS

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***Abstract:*** *This research work is a comparative study of the protein profile of Human Immunodeficiency Virus (HIV) patients that have commenced antiretroviral therapy. The results were grouped into groups of males and females with age grades of 10. The mean values of total proteins for the males are; 86.42 ± 7.82g/l for ages 20-29, 30-39 is 87.85 + 3.12g/l, 40-49 is 72.94 + 9.75g/l, 50-59 is 78.73 ± 4.27g/l and the 60-69 age grade 76.33 ± 2.55g/l. That of the females are 84.71 ± 10.79g/l for ages 20-29, 30-39 has a mean value of 82.89 ± 8.65g/l, 40-49 is 77-88 ± 2.32g/l and 75.69 ± 3.59g/l for ages 50-59. There was no female patient of the age bracket 60-69 on antiretroviral therapy. From the mean values, it is seen that the older patients have a higher rate of deterioration as compared to the younger adults of ages 20-29 and 30-39 that have a higher bioavailability and rate of absorption of the antiretroviral drugs which makes the value of their total protein to fall within the normal range of 62-80g/l because of their increased bioavailability and reduced deterioration rate.*

***Keywords****: Retroviruses, protein, antiretroviral drugs, human immunodeficiency virus (HIV),* patient,

## INTRODUCTION AND LITERATURE REVIEW

A retrovirus is any virus belonging to the viral family *retroviridae.* They are viruses that carry their genetic information as single-stranded RNA. They have the enzyme reverse transcriptase, which forms a DNA copy that is then integrated into the host cell chromosome. They are enveloped viruses possessing a RNA genome and can replicate via a DNA intermediate. The virus replicates as part of the cell's DNA (Nester, 2004).

### Description of the virus

The virus itself stores its nucleic acid in the form of MRNA (including the 5'cap and 3'poly A inside the virion) genome and serves as a means of delivery of that genome into cells it targets as

an obligate parasite, and constitutes the infection (Jawetz et \ aL1995).Once in the host's cell, the RNA strands undergo reverse transcription in the cytosol and are integrated into the host's genome at which point, the retrovirai DNA is referred to as a provirus. When retroviruses have integrated their own genome into the germ-line, their genome is passed on to a following generation. While transcription was classically thought to only occur from DNA to RNA, reverse transcriptase transcribes RNA into DNA. The *retro* in retrovirus refers to this reversal (making DNA from RNA) of the central dogma of Molecular Biology. Reverse transcriptase activity outside of retroviruses has been found in almost all eukaryotes, enabling the generation and insertion of new copies of retrotransposons into the host genome (Nester, 2004).A retrovirus must bring about its own reverse transcriptase in its capsid, otherwise it is unable to utilize the infected cell's enzymes to carry out the task due to the unusual nature of producing DNA from RNA. Retrovirus genomes commonly contain these three open reading frames that encode for proteins that can be found in the mature virus. They are:

- Group-specific antigen (gag) codes for core and structural proteins the virus.

Polymerase *(pol)* codes for reverse transcriptase, protease and integrase and, Envelope *(env)* codes for the retroviral coat proteins.

**HIV as A Retrovirus**

HIV known as the Human Immunodeficiency Virus is a retrovirus, a member of the subfamily lentivirinae. Members of this family induce immunodeficiency and are characterized by a long period of latent infection. (Dozie, 1998). The HIV particle is spherical, about l00nm in diameter with short external spikes on its membranes. The membrane has two layers consisting of lipids and glycoprotein. The glycoprotein layer has two important diagnostic units glycoprotein 41 and glycoprotein 120 (gp 41 and gp 120) projecting from the membrane which is the envelope of the virus. The virus's core includes a protein called p24, the viral RNA that carries the virus genetic information and the enzyme reverse transcriptase that enables the virus to make DNA corresponding to the viral RNA. The main difference between HIV isolates lie in the envelope glycoprotein (Dozie, 1998).

The structure of the HIV genome is more complex than that of transforming retroviruses as they contain accessory genes that interact in different ways with host cell substances and vary with the type of cell infected.

**A Brief History of HIV Virus**

HIV was previously known as the Human T-cell Lymphotropic Virus type III (HTLV-III) /Lymphadenopathy Associated Virus (LAV).

The role of human retroviruses as causative agents in disease dates back to 1978 with the discovery by Robert Gallo of the first human retrovirus which he called, Human T-cell Lymphotropic Virus type I (HTLV-I) (Dozie,1998). The constellation of signs and symptoms associated with these retroviruses came to be known as Acquired Immune Deficiency Syndrome AIDS (Nester, 2004).

HTLV-I causes a rare, highly malignant cancer called; adult T-cell leukemia (ATL) that is endemic in parts of Japan, Africa and Caribbean. Two years after this discovery, Robert Gallo isolated a close relative of HTLV-I which he called the Human T-cell Lymphotropic Virus type II (HTLV-II) from a patient with hairy cell leukemia. Both viruses infect T-lymphocytes and cause disease after a period of latency.

The first AIDS cases were diagnosed in 1981 among young homosexuals in the US by Michael Gottlieb (Dozie, 1998). Although the syndrome was puzzling initially, later it became clear that all its victims suffered from a depletion of a specific subset of T-cell (T4 cells) and as a result became susceptible to pathogens that would be easily controlled by a healthy immune system. Such pathogens are referred to as "opportunistic" pathogens. A variety of hypothesis were advanced to explain this syndrome including; breakdown of the victim’s immune system following repeated exposure to foreign proteins. It seemed more plausible to explain a new syndrome by the appearance of a new infectious agent. To Robert Gallo, who had earlier isolated retroviruses (HTLV-I and HTLV-II), the likeliest agent was a retrovirus. This assumption formed the basis for his research which led to the discovery of the third human retrovirus (Dozie, 1998). The results obtained by Gallo confirmed that LAV and HTLV-III is the same virus. The AIDS virus became known as the HTLV-III/LAV. But the International Commission on the Taxonomy of Viruses changed its name to Human Immunodeficiency Virus (HIV) principally to eliminate the confusion caused by two names for the same entity and to acknowledge that the virus does indeed cause AIDS.

**Life Cycle of HIV Virus**

The life cycle of HIV is similar to that of any other retrovirus. Retroviruses were so named because they reverse what seemed to be the normal flow of genetic information (Dozie, 1998). In cells, the genetic material is DNA. When genes are expressed, the DNA is first transcribed into the messenger RNA (mRNA), which then serves as the template for the production of proteins. The genes of a retrovirus are encoded in RNA, before they can be expressed, the RNA must be converted into DNA. Only then are the viral genes transcribed and translated into proteins in the usual sequence.

HIV must attach to and enter the body's cells to establish infection. When the virus enters the body, it is known as HIV infection, HIV disease implies that the replication of the virus is causing symptoms and the term AIDS refers to the end stage of the HIV disease characterized by unusual Tumors and immunodeficiency (Nester, 2004).

The life cycle begins when the HIV virus binds to the outside of a cell and injects its core. Binding is mediated by proteins on the viral coat and the CD4 receptors and co-receptors on the cell. The core includes two identical strands of RNA as well as structural proteins and enzymes that carry out later steps in the life cycle. The viral RNA is converted into double stranded DNA by the enzyme, reverse transcriptase (Jawetz ,1995).

**Pathogenesis and Pathophysiology of HIV Virus**

The pathogenesis of HIV is quite complex. Following infection with HIV, the virus often replicates abundantly, and free virus appears in the fluid surrounding the brain and spinal cord and in the bloodstream. Fevers, rashes, flu-like symptoms and sometimes neurological complaints can accompany this first wave of HIV replication. Then, within a few weeks, the amount of virus in circulation and the cerebrospinal fluid drops precipitously and the initial symptoms disappear. Though the virus is still present in the T4 lymphocytes, the subset of immune system cells originally thought to be its only target as well as in other classes of immune cells. From two to ten years after the start of this asymptomatic period, replication of the virus flares again and the infection enters its final stage (Dozie, 1998). Previously, it was thought that HIV lies dormant in those immune cells during this asymptomatic phase. It was also believed that an unknown mechanism would trigger the dormant HIV particles to awaken and give rise to the second wave of replicate cycles that will usher in its final assaults in the

patients. Treatments of patients were based on this false picture of the life cycle of the virus. New evidences reveal that there is no dormant phase of the virus infection (Wester, 2004).

The typical course of the HIV infection spans about a decade. Six stages which characterize this course have been described, they are; the primary infection, dissemination of virus to lymphoid organs, clinical latency, induction of HIV expression, clinical disease and death (Jawetz ,1995).

**Mode of Transmission**

Infection with HIV can be initiated through any of the three principal modes of transmission, namely; unsafe sex with an infected person which could be anal, vaginal or oral; via the injection of contaminated blood, blood products and use of unsterile instruments; finally, during the prenatal period of pregnancy (i.e. from mother to baby in the womb) or during parturition through direct mixing of mother's and infant's blood or after birth during breastfeeding. The underlying feature of these three ways of transmission which is fundamental to initiation of infection is the exchange of body fluids which includes blood, semen (including pre-cum), vaginal secretions and breast milk. Blood, semen and vaginal secretions have been reported to have very high concentrations of HIV (Jawetz ,1995).

**Mode of Action**

Following primary infection, viral replication occurs and virus is widely disseminated throughout the body for about 8-12 weeks and the lymphoid organs become seeded. Primary infection is accompanied by symptoms like fevers, rashes, flu-like symptoms and sometimes neurological complaints. There is a significant drop in numbers of circulating CD4 T cells at this early time. An immune response to HIV occurs I week to 3months after infection. This period is called the "window period". The immune response is accompanied by a drop-in plasma viremia and the rebounding of CD4 T cells. However, the immune response is unable to clear this infection completely, and HIV-infected cells persist in the lymph nodes (Dozie,1998). This phase ushers in the period of clinical latency which may last for as long as ten years. Although very few infected cells are in the peripheral blood, it is not a time of true latency as virus-expressing cells are presented and actively replicating in the lymphoid tissues just as the immune system battles to keep the organisms in check. The immune system continues to deteriorate if nothing is done to check the active replication of the virus and finally collapses after a period of time. At this point, the patient develops constitutional symptoms and clinically apparent diseases such as opportunistic infections and neoplasm like Kapos Fs sarcoma. Virus is readily detectable during

the advanced stages of the infection. This typical rise in viral load has been attributed to the degeneration and loss of virus -trapping function of the lymph nodes (Jawetz, 1995)

**Epidemiology**

AIDS was first recognized in the USA in 1981 as a new disease entity in homosexual men. Since then, AIDS had become a worldwide epidemic that continues to expand, afflicting men, women and children and tightening its grip especially in developing countries (Dozie,1998). No continent in the world is presently freeform the lethal reach of the AIDS virus, considering in particular some factors that have continued to hasten the spread of the virus like international movement of infected individuals as a consequence of civil disturbances, tourism and commerce. It was I hypothesized that the rapid dissemination of HIV globally was fostered by massive migration of rural inhabitants to urban centers. Fresh evidence especially from the developing countries shows that the disease has just recently been introduced into the rural population as a consequence of urban-rural mobility pattern with the migrant workers (truck drivers, traders, military men etc) bringing the virus home (Robert, 2004),

**Prevention and Control**

In as much as there is no approved vaccine against HIV infection, infectious HIV persists in samples of blood plasma for at least one week after they are taken from the HIV patients. Most people with HIV disease do not know they are infected and it is advisable to consider all blood samples as potentially containing the virus (Nester, 2004).

Knowing how HIV is transmitted is a powerful weapon against the AIDS epidemic. This weapon can be far more effective than I vaccine or treatment now on the horizon. HIV is not highly contagious, and the risk of contracting and spreading it can be eliminated or markedly reduced by assuming a lifestyle that prevents transmission of the virus. All persons unsure of their HIV status and especially those at increased risk of HIV disease are advised to get tested for HIV. By knowing, those with HIV disease can receive prompt preventive treatment for the infection and cancers that complicates the disease (Lipsky,1996) Antiretroviral treatment of HIV disease before the onset of AIDS shows promise of preventing the complications of immunodeficiency for decades, and it may also reduce transmissibility. Also, HIV positive individuals who know their status can also do their part in preventing transmission. Advances in antiviral treatment mainly results from the development of new medications with different modes

of antiviral action, and use of this medication in combinations known as "cocktails" i.e. combination of reverse transcriptase and protease inhibitors referred to as HAART, "highly active antiretroviral therapy" and are administered based on the replication cycle (Buchbinder,1994)

**Antiretroviral Drugs**

Antiretroviral drugs are medications for the treatment of infection by retroviruses primarily HIV. They are drugs that interfere with the ability of HIV to make new copies of itself inside infected cells. When several such drugs, typically three or four are taken in combination, the approach is known as "highly active antiretroviral therapy" or HAART. There are different classes of antiretroviral drugs that act at different stages of the HIV life cycle. These drugs are broadly classified by the phase of the retrovirus life cycle that the drugs inhibit (Nester, 2004) the classes are:

- Nucleoside and Nucleotide Reverse Transcriptase Inhibitors (NRTI)
- Non- Nucleoside Reverse Transcriptase Inhibitors (nNRTI)
- Protease Inhibitors (Pis) and Integrase Inhibitors.

**Developmental Origin of Antiretroviral Drugs**

Presently there are no approved vaccines for preventing HIV disease. Development of potential HIV vaccines began soon after the discovery of the causative agent. In theory a vaccine could be used either of two ways; one, "preventive vaccine", would be to immunize uninfected individuals against the disease. The other, "therapeutic vaccine", would be to boost the immunity of those already infected with HIV before they become severely immunodeficiency. A successful vaccine must induce both mucosal and bloodstream immunity, because HIV disease is primarily sexually transmitted. The vaccine must also get around the problem of HIV antigenic variability and stimulate cellular and humoral responses against virulence determinants. Moreover, the vaccine has to be safe. An attenuated agent must not be capable of becoming a disease-causing strain, and it must not be oncogenic, meaning cancer-causing. Additionally, the vaccine must not stimulate an autoimmune response, and it must not cause production of "enhancing antibodies" that could aid the passage of HIV into the body's cells. The vaccine should induce neutralizing antibodies against cell-free virions and also prevent direct spread of HIV from a cell to neighboring cells. (Dozie, 1998).

Development of vaccines against HIV disease is taking time and i costing enormous amount of money. At present, there is no vaccine i in sight that could prevent HIV infections entirely (Nester, 2004). The challenge of antiretroviral drug was to develop a drug w*hich* produces very large and long-lasting reductions in viral load id one which affects virus levels both in the blood and the lymph lodes. The hope is that such a drug would provide enough help to body to permit a major restoration of immune health, or at least greatly lengthened period of stable health and halt the loss of CD4 cells, Many scientists argue that unless the virus is brought under control, other efforts at rebuilding immune health will be futile in the long run (Jawetz, 1995).

The actual time to begin treatment with antiretroviral therapy as been a matter of disagreement. Many physicians believe that antiretroviral medication is appropriate immediately upon learning I of the infection, whether or not the CD4 counts is falling or whether symptoms are evident. Other physicians feel that since HIV infection seems dormant for long period during which no major fusible damage occurs; there may be little point in treating an inactive infection. Since recent evidence has shown that the virus is always active and that damage to the immune system is always taking place, starting antiretroviral medication at the earliest possible stage will be more appropriate. With the findings of various scientists, different antiretroviral drugs were developed like Saquinavir which is a protease inhibitor approved in December 1995 to help prevent this worldwide epidemic (Nester, 2004}.

**Pharmacokinetics and Pharmacodynamics of Antiretroviral Drugs**

Pharmacokinetics explains what the body does to a drug, pharmacodynamics on the other hand, explains what the drug does to the body. Concomitantly, they give an insight on the mode of action of the drug.

There are several potential targets for antiretroviral drugs in the viral replication cycle. The major classes of antiretroviral drugs currently used in combination for the treatment of HIV infection, which targets the activities of viral enzymes like Reverse Transcriptase and Protease are explained below though new therapeutic agents are constantly being evaluated (Nester, 2004).

**Reverse Transcriptase Inhibitors**

The first drugs made available for clinical use were inhibitors of the HIV Reverse Transcriptase. Before the virus can be integrated into host cell genome DNA, a copy of the viral RNA has to be formed (proviral DNA). This is regulated by the specific HIV DNA Polymerase; Reverse

Transcriptase. If a DNA copy is not formed, the viral RNA genome becomes susceptible to destruction by cellular enzymes (Jawetz, 1995).

**Protease Inhibitors**

Protease Inhibitors are drugs which act at the late stage of viral Assembly by preventing the production of infectious viruses in Infected cells. The bind competitively to the substrate site of the oral protease. This enzyme is responsible for the post-translational hocussing and cleavage of a large structural core protein during pudding from the infected cell (Nester,2004). Inhibition results in the production of immature virus particles. Specific genotypic mutations in the protease gene can result in high levels of phenotype resistance to individual protease inhibitors and cross resistance. These include Saquinavir tnvirase), Ritonavir (Norvir\ and Indinavir (Crixivan). Nelfinavir (Viracept) is on the verge of being approved for use in HIV infections (Dozie,1998). Some protease inhibitors have been associated with some limitations. Saquinavir has poor bioavailability that may limit its efficacy, Ritonavir has been associated with gastrointestinal intolerance and with peripheral neuropathy, which has led to poor compliance in some patients.

**Integrase Inhibitors**

Integrase inhibitors inhibit the enzyme integrase which is possible for the integration of viral DNA into the DNA of the cited cell. There are several integrase inhibitors currently under and Raltegravir is the first to receive approval in October )7 (Encyclopedia Britannica,2007). Entry inhibitors (or fusion ubitors) interfere with binding, fusion and entry of H1V-1 to the cell by blocking one of several targets. Maraviroc and fuvirtide are the two currently available agents in this class. Maturation inhibitors inhibit the last step in gag processing in such the viral capsid polyprotein is cleaved, thereby blocking the Aversion of the polyprotein into the mature capsid protein. There no drugs in this class currently available, though some are Bider investigations (Nester, 2004). Most antiretroviral drugs given in fixed dose combinations which are multiple antiretroviral drugs combined into a single pill.

**Antiretroviral Drug Resistance**

Reverse Transcriptase which is the mode of replication of HIV is lacks the visual proofreading of DNA replication, which makes this kind of virus mutate very often. This enables the virus to grow resistant to antiviral Pharmaceuticals quickly, and impedes the development of effective vaccines and inhibitors for the retrovirus, "Highly Active

**Factors Determining When to Start Antiretroviral Therapy**

- Risk of clinical disease progression (CD4 count, viral load)
- Willingness of patient to start therapy
- Clinical effectiveness of combination therapy -Ability and motivation of patient to adhere to therapy
- Drug toxicity profile
- Pill burden and dosing schedule
- Transmitted drug resistance
- Future therapy options
- Likelihood of drug resistance
- Drug-drug interactions

**Antiretroviral Drug Toxicity and Adverse Effects**

The tolerability and side effects of a combination regimen is very important in determining the antiviral response. In clinical practice, 40-50% of patients will not have sustained falls in plasma viral load by one year of therapy and a major factor contributing to this is poor tolerability (Dozie, 1998).

Toxic effects of the medication are a limitation of anti-HlV therapy. For instance, AZT like Zidovudine can cause anemia, low white blood cell count, vomiting, fatigue, headache, muscle and liver damage. Painful peripheral nerve injury, inflammation of the pancreas, rash, mouth and esophagus ulceration and fever are side effects of other nucleoside reverse transcriptase inhibitors. Non-nucleoside reverse transcriptase inhibitors like nevirapine and efavirenz could cause rash, dysphoria, mood changes, and hepatitis (Nester, 2004). Also, Indinavir may be responsible for kidney stone formation, and Ritonavir, another protease inhibitor, causes nausea and diarrhea. Diabetes mellitus is another potential side effect of protease inhibitor.

Finally, more comparative studies on the replicative cycle of the HIV genome, and the combination therapy by the drug designers are in progress to aid reduce the drug toxicity and improve the use of HAART and combined therapy.

**Total Protein**

Total plasma proteins are made up of albumin, globulin, and fibrinogen. While total serum proteins are made up of albumin and globulin. The reference range for total protein estimated in the serum is 62-80g/l though this excludes fibrinogen which is included in plasma proteins. The

measurement of the total protein content of the plasma may be used to assess the degree of hydration of the patient and is required if the calculation of the total globulin is to be made (Baron, 1998). Most of the causes of low total protein levels (below 60g/l) are due to reduced albumin concentrations or more rarely to severe immunoglobulin deficiency. An increase in the total protein concentration can occur (greater than 80g/l) when there is prolonged venous stasis during venepuncture, or when a person is dehydrated (Cheesebrough, 2005).

**Aim of Study**

- To ascertain the protein profile of HIV patients on anti-retroviral therapy.
- To evaluate the nature of the progress of irregularities in the biochemistry of the HIV patients such as change in the plasma proteins as a result of changes in the CD4 count and viral load.

## 2. RESEARCH MATERIALS AND METHODOLOGY

**Materials**

These encompass the physical objects which were used as apparatus and the reagents which were used during the experimental course of this work. Such materials include:

- ✓ **Test-tubes:** These are glass tubes closed at one end which is used to store the collected serum samples.
- ✓ **Test-tube rack:** This is a wooden stand with holes in which the |test-tubes are kept.
- ✓ **Micropipette:** This is a calibrated narrow tube which is very small is compared to the normal sized pipette hence the name micro. It is used to collect the separated serum from the red blood cells.
- ✓ **Centrifuge:** This is used to spin the blood samples to separate the serum from the erythrocytes.
- ✓ **Spectrophotometer:** This is an instrument used to read the absorbance of the mixture after the reagent has been added, it is usually zeroed with the blank

**Reagents**

**Biuret reagent:** This is the reagent used to test for total protein I in a sample. It is made up of sodium hydroxide, sodium-potassium tartrate, cupric sulphate and potassium iodide in different I concentrations. The cupric ions react with the protein in an alkaline medium to form a blue coloured complex.

- ✓ **Bromocresol green (BCG):** This is the reagent used to determine serum albumin concentration of the blood sample. It is 3mposed of succinate buffer at $p^H$ 4,2, and Bromocresol green. The albumin standard reagent and the total protein standard regent were also provided.

**Determination of Total Protein**

**Principle:** Protein/peptide bonds present in serum forms a violet coloured complex when reacted with cupric ions in an alkaline solution. The intensity of the violet colour is proportional to the amount of protein present when compared to a solution with known protein concentration, The reaction sequence employed in the assay of total proteins is as I follow: Protein + $Cu^{2+}$ Alkaline $p^{H}$ Cu-protein complex

**Reagent/Method Used:** ⟶ Biuret reagent/method

**Procedure**

- The total protein standard reagent of concentration 60g/l was measured using a micro pipette of 50($\mu$l to a clean dry test tube and 2.5ml of the biuret reagent was added to the test tube.
- A blue coloured complex was observed.
- The mixture was mixed and allowed to stand for lOmins at room temperature.
- The procedure was repeated using the test serum samples of the different patients, that of the quality control and distilled water which was used as the blank were also carried out using different test tubes. The same volume of the biuret reagent i.e. 2.5ml was used in each case.
- A bluish-coloured complex of different colour intensities was observed in each case.
- The absorbance readings of the different test tubes were taken from the spectrophotometer which was set at zero with the blank and the absorbance readings of the standard, quality control and test sample were read at a wavelength of about 540nm. The serum protein concentration was calculated using the formula:

$$\text{Total protein} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \frac{\text{Standard concentration}}{1}$$

Where the standard concentration is given as 60g/l and the normal total protein range is 60-82g/l

**Technique**

The test tubes are labeled as protein standard, quality control, test sample and blank (distilled water).

| | Standard | QC | Test | Blank |
|---|---|---|---|---|
| Reagent | 2.5ml | 2.5ml | 2.5ml | 2.5ml |
| Standard(60g/l) | 50^1 | - | - | - |
| Quality control | - | 50$\mu$1 | - | - |
| Test sample | - | - | 50$\mu$l | - |
| Distilled water | - | - | - | 50$\mu$1 |

**Determination of Serum Albumin**

**Principle:** Albumin present in serum binds specifically with bromocresol green at a $p^H$ of about 4.2 to form a green coloured complex which can be read from the spectrophotometer a wavelength of about 600nm and the absorbance being directly proportional to the concentration of albumin in the sample.

**Reagents/method used:** Bromocresol green reagent/method.

**Procedure**

Four test tubes were labeled as follows; blank, standard, control serum and test serum.
Bromocresol green reagent of volume 3.0ml was measured using the pipette into each test tube.- Distilled water of volume 20|il was added to the test tube labeled blank, 20^1 of the standard reagent was added to the test tube j labeled standard, and the same volumes of each sample were also added to the test tubes labeled control serum and test serum respectively.
The different test tubes were mixed well and frothing of the solution was avoided.
The test tubes were then allowed to stand for about 10 minutes at room temperature.
The same procedure was repeated for the different patient's sera and each was mixed well and allowed to stand for about comminutes at room temperature.
The absorbance readings were read from the spectrophotometer at a wavelength of about 600nm and the instrument was zeroed with the blank.

The concentration of albumin in the control and patient's sera was calculated thus;

$$\text{Albumin (g/1)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \frac{\text{Standard concentration}}{1}$$

Where the standard concentration of the albumin is 36g/l and the normal range 36-50g/l.

**Technique:**

The test tubes were labeled; albumin standard, quality control, test sample and blank as follows;

| | Standard | Control | Test sample | Blank |
|---|---|---|---|---|
| Reagent | 3.0ml | 3.0ml | 3.0ml | 3.0ml |
| Standard | 20$\mu$l | - | - | - |
| Control | - | 20/$\mu$l | - | - |
| Test serum | - | - | 20$\mu$1 | - |
| Blank | - | - | - | 20$\mu$1 |

**Determination of Globulins**

The globulin values can be determined by subtracting the albumin value from the total protein value. Serum globulin, g/1 = total protein - albumin (g/I) Normal range is about 20 - 36 g/I Albumin/Globulin ratio = Serum albumin, g/J Scrum globulin, g/1

1. **RESULT PRESENTATION**

The presentation of the already analyzed I data gotten in the course of the research. The mean values were calculated by grouping the results in age brackets with ranges of 10 and also based on the sexes of the patients. The result of the total j protein and albumin are presented below;

**Table 3.1 Total protein mean values for the male patients.**

| Age Grade | No of Males | Mean and S.D |
|---|---|---|
| 20-29 | 4 | 86.45 ± 7,82 g/1 |
| 30-39 | 3 | 87.85±3.12g/l |
| 40 -49 | 2 | 72.94 ±9.75 g/1 |
| 50- 59 | 3 | 78.73 ±4.27 g/1 |
| 60-69 | 2 | 76.33 ±2.55 g/1 |

**Table 3.2 Total protein mean values for the female patients,**

| Age grade | No of females | Mean and S.D |
|---|---|---|
| 20- 29 | 7 | 84.71 ± 10.79 g/1 |
| 30-39 | 4 | 82.89 + 8.65 g/1 |
| 40-49 | 3 | 77.88 ±2.32 g/1 |
| 50-59 | 2 | 75.69 ±3. 59 g/1 |
| 60-69 | 0 | |

**TableS.3 Albumin means values for the male patients.**

| Age grade | No of males | Mean and S.D |
|---|---|---|
| 20 - 29 | 4 | 45.36 ± 8.35 g/1 |
| 30-39 | 3 | 51. 98 ±4.70 g/1 |
| 40-49 | 2 | 38,42±3.87g/1 |
| 50- 59 | 3 | 40.68 ± 2.29 g/1 |
| 60-69 | 2 | 40.68 ± 0.32 g/1 |

**Table 3.4 Albumin mean values for the female patients.**

| Age grade | No of females | Mean and S.D |
|---|---|---|
| 20- 29 | 7 | 47.32 ±7.30 g/1 |
| 30- 39 | 4 | 45.85 ± 8.76 g/1 |
| 40- 49 | 3 | 41.81 ± 5.55 g/1 |
| 50-59 | 2 | 37.29 ± 2. 92 g/1 |
| 60-69 | 0 | - |

Where S.D means standard deviation and the normal range for the total protein is 62 - 80 g/1 while that of albumin is 36 - 50 g/1.

**DISCUSSION AND CONCLUSION**

From the research work and studies carried out in the previous chapters, it has been invariably shown that the HIV virus increases the viral load of the patient which suppresses the $CD4^+$ cell count and plasma protein contents. It is seen from the results which has been grouped to different age brackets that the male patients of the age grade 20-29 have a total protein value of 86.45 ± 7.82 g/1 though the females of the same age bracket have a slightly lower mean value of 84.71 ± 10.79 g/1 though both values are within the normal range of 62-80 g/1 due to the administration of antiretroviral therapy on the patients. The male of age bracket 30-39 also have a mean value of 87.85 ± 3.12 g/1 which is higher than that of the females of the same age bracket which is 82.89 ± 8.65 g/1. Also, the males of age bracket 40 - 49 have a much lower value of 72.94 ± 9.75 g/1 than the values above though the value is also within the normal range of 62-80 g/1. Their female counter parts have a mean value of 77.88 ± 2.32 g/1. The male of the 50-59 age grades have a mean value of 78.73 ± 4.27 g/1, while the female counterparts have a mean value of 75.69 ± 3.59 g/1. Those within the age bracket 60-69 have a mean value of 76.33 ± 2.55 g/1 and there is no value for the females of that age bracket because there was no female of that age bracket on antiretroviral therapy.

From the results discussed above, it shows that the plasma protein content can be normalized by the administration of antiretroviral drugs and it is seen that the younger adults of age bracket 20-29 and 30-39 have very high values at those ages when compared with | ages 50-59 and 60-69. This indicates that the rate of absorption I and distribution of drugs is higher at those ages due to a better I functionality of the organs which declines progressively as the age I of the patient progresses. Also, the bioavailability is high at those I ages at which the rate of absorption is high. These factors in turn affects the values of the plasma proteins at those ages which indicates that

age is an important determinant in the normalization I of the $CD4^+$ cell count and plasma protein contents of HIV patients.

Studies by Mocroft in 2007 of the Royal Free and University College Medical School, London shows that the decline in CD4+ lymphocytes and plasma proteins occurs at different rates in patients with HIV virus. A longer duration of HIV infection and a higher level of viral replication, represented by the viral load are associated with a lower $CD4^+$ lymphocytes count, though the interrelationship between these variables is still not elaborately known. Although older patients and a high level of plasma viraemia, have been associated with a faster fall in CD4 T-cells due to the higher deterioration rate of older patients and their slower response to antiretroviral therapy.

Conclusively, in as much as the combined antiretroviral therapy does not cure AIDS, it reduces the viral load to a great I extent but does not eliminate HIV provirus hidden in the host cell I genomes. From the results discussed above, it is seen that for the I female patients, the mean values are; 84.71 ± 10.79 g/1, 82.89 ± I 8.65 g/1, 77.88 ± 2.32 g/1 and 75.69 ± 3.59 g/1 for the age brackets 20-29, 30-39, 40-49 and 50-59 respectively which indicates that the younger female adults respond more to the antiretroviral therapy as seen by the mean values which decreases progressively as the age increases. The result is similar for the male patients of the same age brackets though there is a variation in the male patients of age bracket 40-49 with a mean value of 72.94 ± 9.75 g/1, though the reason for this variation is not exclusively known since the ages 50-59 and 60-69 have mean values of 78.73 + 4.27 g/1 and 76.33 ± 2.55 g/1 which is greater than the value of the age bracket 40-49 though the are all within the normal range of 62-80 g/1. Therefore, it is seen that antiretroviral therapy goes a long way to invariably normalize the patient's $CD4^+$ cell count and plasma proteins and the medication should commence as soon as the HIV disease is detected to enhance the effects of the antiretroviral therapy and to control deterioration of the patient.

**RECOMMENDATION**

I recommend that further studies should be carried out on the interrelationship between the duration of the administration of antiretroviral therapy and the normalization of the $CD4^+$ cell count and plasma proteins. These studies will enable the physicians and the HIV patients to know the definite time to commence the administration of the antiretroviral therapy and thus reduce the immune system dysfunction caused by HIV and also slow down or stop the replication of HIV as soon as and as much as possible. In addition, I recommend that drug designers should carry out further studies on the pathogenesis of the HIV virus and the adverse effects of the antiretroviral drugs to improve the timing of the drugs to aid reduce drug resistance and to regulate the toxicity and adverse effects of antiretroviral therapy.

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