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THE EFFECT OF AGE, GENDER AND LOCATION OF RESIDENCE ON CD4 COUNTS RESPONSE TO ARV THERAPY IN PATIENTS WHO ATTEND NYAGATARE HOSPITAL IN VCT SERVICE

A dissertation submitted and presented in partial fulfillment of requirements for the award of Bachelor's Degree of sciences in Biotechnologies

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ORIGINAL STATEMENT

"I hereby declare that this dissertation submitted in partial fulfilment of the requirement for the award of Bachelor's degree of Science in Biotechnology at INES, is my original work and has not previously been submitted elsewhere. Also, I do declare that a complete list of references is provided indicating all the sources of information quoted or cited.

Edith TUMWEBAZE

DEDICATION

Our parents;

Our brothers;

Our sisters and

Our relatives.

This work is dedicated.

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Edith TUMWEBAZE

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ABSTRACT

Measurement of CD4 is essential for assessing the progress of human immunodeficiency virus (HIV) disease. This parameter is also used in clinical staging, epidemiological studies, and decisions regarding prophylactic therapies against opportunistic infections. CD4 cell counts, alongside other parameters, are of importance in the monitoring of HIV/AIDS immune response function. As HIV/AIDS patients' access to care increases, there is need to determine the factors that affect CD4 counts in order to advise patients on practices that increase their CD4 counts in conjunction with ARV therapy. There is evidence in literature of significant geographical, racial, age and gender difference responses in CD4 count of patients under ARV therapy. The objective of this study was to assess effect of patient specific factors (age, gender, location of residence, changing of the drug, alcohol intake and type of drug taken) on the increase in CD4 counts during the ARV therapy for patients who attended Nyagatare Hospital in VCT service. A random sample of 150 HIV^+ patients who attend VCT service were selected interviewed using a questionnaire.

The respondents were asked about their ARV therapy history and CD4 counts, where they resided, age, gender and alcohol intake. The CD4 count response (CD4 count after ARV therapy minus CD4 count before ARV therapy) was computed. Excel was used to compare the mean CD4 count responses of young (18-35 years of age) and old (35+ years of age), male and female, urban and rural location of residence. CD4 count responses were correlated to age, location and gender. There was a significant difference in the CD4 count responses between young and old HIV^+ patients. Other factors (gender and location) did not significantly affect CD 4 count responses to ARV therapy. There was a significant difference in mean between age and CD4 count response to ARV therapy. Other factors (gender and location) had no significant mean difference to CD4 count responses. It is recommended that testing for HIV infection be started early at a young age so that patients can begin ARV treatment early when they are young and likely to exhibit higher CD4 count increases in response to ARV therapy.

RÉSUMÉ

La mesure de CD4 est essentielle pour l'évaluation du progrès du virus d'immunodéficience humaine. Ce paramètre est aussi utilisé dans le stade Clinique, étude épidémiologique et dans les décisions prophylactiques et thérapeutiques contre l'infection opportuniste.
Le compte de cellules de CD4 et autres paramètres sont importants dans le suivi de réponse de VIH/SIDA de fonction immunitaire. Si le suivi du patient de VIH augmente, il ya aussi le besoin de déterminer le facteur qui affecte le CD4 pour conseiller le patient dans les pratiques qui augmentent le CD4 avec la combinaison de traitement de ARV. La littérature prouve que le signe géographique, racial, d'âge et de genre se différent du point de vue réponse sur le CD4 sous le traitement d'ARV. Les objectifs d'étude est d'effectuer le facteur spécifique qui affecte le patient (âge, genre et résidence) sur l'augmentation de CD4 sous traitement de ARV par le patient qui fréquente l'hôpital de Nyagatare dans le service de VCT. Selon l'échantillonnage aléatoire, la population de 150 personnes possédant le VIH qui fréquentent le service de VCT ont été sélectionnées pour l'interview en utilisant le questionnaire.

Les répondants ont été demandés sur l'histoire de leur traitement et leur CD4 où ils habitent, âge, genre et la consommation de l'alcool. La réponse de CD4 (CD4 après le traitement de ARV moins CD4 avant le traitement de ARV) a été analysée. Le logiciel Excel a été utilisé pour comparer la moyenne de la jeunesse (18-35 ans) et les adultes (>35 ans), mâle et femelle, en ville et au village. Le CD4 a été comparé avec l'âge, genre et résidence. Il y a une différence importante entre l'augmentation de CD4 pour la jeunesse et adulte. Autre facteur (genre et résidence) n'avez pas une différence importante. Nous avons recommandé que la population doit se faire dépister bien avant et que les patients commencent le traitement de ARV pour augmenter leurs CD4.

List of Abbreviations

AIDS: Acquired Immunodeficiency Syndrome
ALAT: Alanine amino Transferase
ARV: Antiretroviral therapy
ASAT: Aspartate Amino Transferase
CCR5: Chemokinine Receptor 5
CDC: Centre for Disease Control
CD4: Cluster of Differentiation 4
FACS: Flow Activated Cell sorting
FBC: Full Blood Count
HAART: Highly Active Antiretroviral Treatment
HIV: Human Immunodeficiency Virus
INES: Institute d'Enseignement Supérieur de Ruhengeri
PCP: Pneumocystis Carinii pneumonia
PEPFAR: President's Emergency Plan for AIDS Relief
PMTCT: Prevention of Mother to Child Transmission
SPSS: Statistical Package for Social Scientist
TB: Tuberculosis
TRAC: Treatment and Research on AIDS Centre
USA: United States of America
VCT: Voluntary Counselling and Testing
VDRL: Venereal Disease Research Laboratories
WWW: world word web

GENERAL INTRODUCTION

1. Background to the study

The World Health Organisation has estimated that more than 90% of a world total of 30 to 40 million cases of the human immunodeficiency virus infection by the year 2000 will be in developing countries (Global Program on AIDS, 1993). Present knowledge concerning the staging of the disease, monitoring of disease progression and initiation of therapeutic regimens depends heavily on determination of peripheral lymphocytes subpopulations (National Institute of Health, 1990)

Measurements of CD4 lymphocytes are essential for assessing HIV disease course, clinical staging, epidemiological studies and decisions regarding prophylactic therapies against opportunistic infections (CDC and Prevention, 1992). Only in industrialized countries have it been feasible to routinely monitor CD4 lymphocytes subsets during routine HIV clinical care.

The adult prevalence of HIV infection in Rwanda is currently estimated to 3% (national HIV sero-surveillance report, (2005 TO 2009), which translates to 300 000 infected persons in a country with a population of 8 million. In 2011, The prevalence of HIV in Rwanda is 3%, 7.3% in Kigali, 2.2% in villages, 5% males, 8.2% females and 169 200 people are infected. Females are more infected than males and their age ranges between 15 and 24. (TRAC, television broadcast on 17/08/2011). Fortunately, both local and international efforts, especially the US President's Emergency Plan for AIDS Relief (PEPFAR) and the Global Fund, have recently been directed towards the provision of antiretroviral therapy for the estimated 120.000 persons living with HIV/AIDS in the country.

CD4 cell counts, alongside other parameters, are of central importance in the monitoring of the immune function. In Nigeria, country-specific reference ranges for haematological parameters have been determined, (Azikiwe, 1984), CD4 cell reference values are not available from West African populations this means that the CD4 values which are in use here in Africa are from the studies made from the Caucasians. Values from text books and other publications based on studies in western countries are largely employed for clinical decision making.

However, there is evidence in the literature of significant geographical and racial differences in these parameters. For example, lower CD4 cell count have been recorded for Asians (Lee et al..,1996) than for Caucasians, and studies in African population have shown mean CD4 cell counts in healthy Ethiopians (Tsegaya et al..,1999) that are markedly lower than in Ugandans (Tugume et al..,1995) and Tanzanians (Brubaker et al..,1996). Normal reference ranges of lymphocytes subpopulations in adults have been established for Caucasians but little data is presently available for immunophenotype assessment of Rwandan adults.

However, there has been noted out by many scientists that there are factors which affects CD4 count. Age do affect CD4 count where younger people increase their CD4 count than older people and this is due to a decrease of the capacity of the thymus in production of CD4 in older people while the younger ones still have the capacity to produce CD4 (Roberto M et al, 2000).Scientists talked alot on gender and studies showed out that females increase their CD4 than males (Moree et al, 2001), but the exact reason of this difference is not given. Location affects the CD4 count where people in urban areas increase their CD4 count than people in rural areas and the reason for this is due to access to treatment, care and follows up of urban people than village people (Susan et al, 2001). Factors other than HIV can affect CD4 count including infections, time of day, smoking, stress, recreational drug use, infection route, genetics, pregnancy, repeated exposure to HIV, nutrition and experience of health care providers.

2. Problem statement

AIDS is a world pandemic disease which is a life threatening and kills a lot of people.

HIV is a major cause of immunodeficiency diseases like AIDS and this disease has no treatment but the increase in CD4 can make a person to live longer with AIDS and in order to increase CD4, there is a need for HIV+ patients to take ARV drugs but factor like age, gender and location affect the increase of CD4 count and these cells are the target of HIV and they help to fight infection so the effect of these factors slow down the response to the ARV therapy and the improvement of immune system and hence affects the health of HIV^+ patients.

3. Motivation of the study

During clinical training at university teaching hospital of Butare, it was noted that patients on ARV therapy responded differently to the treatment and no studies have so far done to determine the factors which caused these HIV+ patients to respond differently to the ARV therapy

4. Significance of the study

The study will generate information on which factors (age, gender and location of residence) affect the increase in CD4 counts. The information generated from the study will be used to generate recommendations on practices that increase the response to ARV therapy.

5. Hypothesis

CD4 counts increase in response to ARV therapy is more in young than old patients, increase depending on gender of the patient and increase more in people living in urban areas than rural areas.

6. Objectives

6.1 Main objective

To assess the impact of patient specific factors on CD4 counts in patients on ARV therapy from VCT service of Nyagatare Hospital

6.2 Specific objective

To determine whether CD4 increase according to age, gender and location of residence after ARV therapy cocktail.

1.7 Subdivision of the study

A part from the introduction, our study was divided into 4 chapters:

Chapter one is the literature review.

Chapter two is the methodology which highlights the materials and methods used. These include the techniques, tests and procedures used for laboratory diagnosis.

Chapter three will focus on the presentation of the results and discussions

Chapter four is the conclusions and recommendations.

CHAPTER ONE: LITERATURE REVIEW

1.1. Definitions of CD4

A type of T cell involved in protecting against viral, fungal, and protozoal infections. These cells normally orchestrate the immune response, signaling other cells in the immune system to perform their special functions, also known as T helper cells HIV's preferred targets, (Ivan roittt, 1984).

1.1.1. Role of CD4 in HIV infection.

Human immunodeficiency virus (HIV) infection of permissive cells is initiated by virus binding to its cellular receptor, the CD4 molecule using recombinant, soluble CD4 (sCD4) as a receptor mimic, have investigated the molecular interactions taking place during and subsequent to binding which result in virus-cell and cell-cell fusion. Binding of CD4 to the surface of HIV-1-infected cells induces conformational changes in the envelope glycoproteins, as demonstrated by the modulation of monoclonal antibody (mAb) epitopes on both gp120 and the viral transmembrane glycoprotein, gp41. The same studies carried out on the related immunodeficiency viruses HIV-2 and SIV yield contrasting results, implying an entry pathway subtly different from HIV-1, and suggesting a mechanism for the resistance of HIV-2 and SIV to sCD4 neutralisation. We have analysed the glycoprotein interactions taking place at the interface between CD4+ and HIV-infected cells in the process of fusing. Both CD4 and gp120 are rapidly clustered at the junction between fusing cells, and cryptic gp41 epitopes are exposed at this interface. We propose that these molecular rearrangements are important steps in the virus-cell fusion process. In addition, we present evidence demonstrating that the high affinity binding site on CD4 for gp120, the CDR-2 loop, is necessary and sufficient for inducing post-binding events leading to HIV-cell membrane fusion, Pat R Bucy et al (1999).

1.1.2. Normal ranges of CD4

Different authors give different values like Smith et al,(2003) give the normal value of CD4 as 500-1600 cells/µl while US department ,(2002) and other books give 500-1200 cells/µl which is the value being used here in Rwanda.

1.2. Antiretroviral therapy

1.2.1. Antiretroviral drugs are medications for the treatment of infection by retroviruses, primarily HIV. When several such drugs, typically three or four, are taken in combination, the approach is known as Highly Active Antiretroviral Therapy, or HAART. The American National Institutes of Health and other organizations recommend offering antiretroviral treatment to all patients with AIDS. Because of the complexity of selecting and following a regimen, the severity of the side-effects and the importance of compliance to prevent viral resistance, such organizations emphasize the importance of involving patients in therapy choices, and recommend analyzing the risks and the potential benefits to patients with low viral loads, Centers for disease control and prevention, (1992).

Classes of antiretroviral drugs

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

- Nucleoside and nucleotide reverse transcriptase inhibitors (NRTI) inhibit reverse transcription by being incorporated into the newly synthesized viral DNA strand as a faulty nucleotide. This causes a chemical reaction resulting in DNA chain termination.
- Non-nucleoside reverse transcriptase inhibitors (NNRTI) inhibit reverse transcriptase directly by binding to the enzyme and interfering with its function.
- Protease inhibitors (PIs) target viral assembly by inhibiting the activity of protease, an enzyme used by HIV to cleave nascent proteins for the final assembly of new virons.
- Integrase inhibitors inhibit the enzyme integrase, which is responsible for integration of viral DNA into the DNA of the infected cell. There are several integrase inhibitors currently under clinical trial, and raltegravir became the first to receive FDA approval in October 2007.
- Entry inhibitors (or fusion inhibitors) interfere with binding, fusion and entry of HIV-1 to the host cell by blocking one of several targets. Maraviroc and enfuvirtide are the two currently available agents in this class.

- CCR5 receptor antagonists are the first antiretroviral drugs which do not target the virus directly. Instead, they bind to the CCR5 receptor on the surface of the T-Cell and block viral attachment to the cell. Most strains of HIV attach to T-Cells using the CCR5 receptor. If HIV cannot attach to the cell, it cannot gain entry to replicate.
- Maturation inhibitors inhibit the last step in gag processing in which the viral capsid polyprotein is cleaved, thereby blocking the conversion of the polyprotein into the mature capsid protein (p24). Because these viral particles have a defective core, the virions released consist mainly of non-infectious particles. Alpha interferon is a currently available agent in this class. Two additional inhibitors under investigation are bevirimat and Vivecon, Centers for disease control and prevention, (1992).

1.2.3. Initiation of antiretroviral therapy

1.2.3.1. World wide and in Africa

Antiretroviral drug treatment guidelines have changed over time. Prior to 1987, no antiretroviral drugs were available and treatment consisted of treating complications from the immunodeficiency. After antiretroviral medications were introduced, most clinicians agreed that HIV positive patients with low CD4 counts should be treated, but no consensus formed as to whether to treat patients with high CD4 counts.

In 1995, David Ho promoted a "hit hard, hit early" approach with aggressive treatment with multiple antiretrovirals early in the course of the infection. Later reviews noted that this approach of "hit hard, hit early" ran significant risks of increasing side effects and development of multidrug resistance, and this approach was largely abandoned.

The timing of when to initiate therapy has continued to be a core controversy within the medical community. The development of a stable consensus is hampered by the lack of randomized controlled studies with many guidelines and consensus statements basing their recommendations on observational studies. More recently, the trend has been in favor of earlier treatment of asymptomatic HIV patients, with more studies analyzing various treatment regimens in progress.

There is a consensus among experts that, once initiated, antiretroviral therapy should never be stopped. This is because the selection pressure of incomplete suppression of viral replication in

the presence of drug therapy causes the more drug sensitive strains to be selectively inhibited. This allows the drug resistant strains to become dominant. This in turn makes it harder to treat the infected individual as well as anyone else they infect. HIV drug treatment is generally recommended when the CD4 test shows fewer than 350cells/mm^3.

World Health Organization (WHO) 2010 guidelines recommend starting treatment for all patients with CD4 counts of 350cells/mm^3in all countries. Although most resource-limited countries aim to follow these guidelines, a number still observe the WHO's 2006 guidelines, which recommend starting treatment at less than 200 cells/mm^3, (Universal access to AIDS treatment).

Some countries may have treatment guidelines which differ from WHO recommendations. For example, although USA treatment guidelines state that treatment should be initiated in all patients with a CD4 count <350 cells/mm^3 they also recommend treatment for patients with a CD4 count between 350 and 500 cells/mm^3. If there are complications such as if the patient has hepatitis B, an AIDS-defining illness or pregnancy, guidelines usually recommend that treatment is started earlier.

- **Side effects of ARV**

Adverse effects of antiretroviral drugs vary by drug, by ethnicity, by individual, and by interaction with other drugs, including alcohol. Hypersensitivity to some drugs may also occur in some individuals. The following list is not complete, but includes several of the adverse effects experienced by patients taking some antiretroviral drugs:

- Abdominal pain (Ritonavir)
- Anemia (AZT)
- Diarrhea (Abacavir)
- Dizziness (Vertigo)
- Headache (3TC overdose)
- Hepatitis
- Hyperbilirubinemia
- Hypercholesterolemia and etc.

- **WHO clinical staging**

In poorer countries, CD4 testing may be unavailable due to the expense of the equipment. In these cases, there is a method of describing the different stages of HIV disease based on clinical symptoms, known as the WHO staging system for HIV disease.

Where a patient is showing signs of stage 1 and 2 they should not start treatment. However, if they are showing signs of WHO clinical stages 3 or 4 they should start treatment. Clinical stages 3 and 4 are identified by the emergence of certain opportunistic infections (such as PCP) and cancers, which a healthy immune system would normally fight off.

- **Basic clinical assessment**

Before a person starts treatment, a basic clinical assessment should also be carried out. This assessment determines, for example, existing medical conditions (such as hepatitis, TB, pregnancy, injecting drug use and major psychiatric illness), whether or not the individual is currently taking medications (including traditional and herbal HIV medications), their weight measurement, and a patient's readiness for therapy. Treatment should only be started once the person is ready. A lot of commitment is needed, since following a drug regime can be quite demanding and in most circumstances, the treatment will have to be taken every day for life.

Once it is decided that treatment should be started, doctors will give advice about the various HIV drugs and combinations available and which might be most suitable.

1.2.3.2. Initiation of ARV drugs in Rwanda

The patients who attend VCT service are from the following services

1- VCT
2- PMTCT

After finding that they are HIV positive, they are referred to VCT/ARV service for CD4 controls.

They are registered in the register of the service and then the file for the patient is made.

CD4 count is done and if the patient is found to have the CD4 ≤ 250 cells/µl of blood, the patient is eligible to the treatment. Before starting the treatment, there is a pre-antiretroviral treatment counseling and adherence that is all precautions concerning the treatment (The term adherence means taking the drugs exactly as described. This includes taking all of the medication at the right time and exactly as the directions state. It also means ensuring that there will be no interactions with other drugs being taken.). And also laboratory tests are done which are:

1-FBC

2-ASAT

3-ALAT

4-CREATININE

5-Pregnancy test

6-VDRL

After getting the results and after finding the results feasible, the patient starts the treatment. The patient is followed for two weeks and then the above laboratory tests are repeated to see whether the treatment is not causing harm to the patient. Then every month, the patient is attending the hospital to check the kilos, clinical follow up and to treat any disease which has attacked the patient. And the CD4 are checked every six months to see whether the ARV therapy is responding. If the ARVs are not responding, the patient is shifted to the second line of treatment and there is no third line in Rwanda.

1.2.3.3. ARV therapies which are mostly used in Rwanda

- **First line**

1. TDF 300mg (Tenofovir) + 3TC 300mg (Lamividine) and 1 tablet is taken per day.

2. AZT 300mg (Diovir-N) + Lamividine 300mg and 1 tablet is taken two times a day.

3. AZT 300mg (Diovir-N) + NEV 200mg (Nevirapine) and 1 tablet is taken two times a day.

4. Abacavir 300 mg and 1 tablet is taken two times a day.

5. Effavirenz 600 mg and 1 tablet is taken a day.

When the first line fails on a patient, he or she is shifted to the second line.

- **Second line**

1. Kaletra 125 mg and 2 tablets are taken two times a day. Kaletra is combined with one of the drug in the first line according to the doctor's choice for the patient.

There is no third line in Rwanda though developed countries have them and there are some drugs used here in Rwanda while other countries do not use them, this is due to economy of the country and accessibility of getting the drugs.(retrieved from Rwanda's guidance on initiation of ARV therapy/www.tracnet.gov.rw)

1.2.3.4. Factors affecting the CD4 counts

1. Age

Older Patients: The older the HIV patient, the faster he or she is likely to progress to AIDS. The effect is most apparent in patients older than 40. Researchers estimate that the risk of developing AIDS increases 27-55% every ten years. According to a meta-analysis of 38 studies that involved more than 13000 HIV patients, age and time since diagnosis were significant factors in determining the rate of HIV progression. Patients who developed HIV antibodies between the age of 15 and 24 lived an average of 12.5 additional years. These patients progressed to AIDS

after an average of 11 years. Patients who developed HIV antibodies between the age of 45 and 54 lived an average of 7.9 additional years. These patients progressed to AIDS after an average of 7.7 years (ARV therapy use in adolescents and adults, 2011).

While the exact reason of this is not known, some researchers suggest that older patients have a decreased ability to replace the CD4 T-cells that HIV infects and destroys. It is also unclear whether this is a result of the thymus gland's inability to produce new CD4-T cells. Others suggest that older patients may have lower levels of chemokines, white blood cells that help fight off HIV, (ARV therapy use in adolescents and adults, 2011).

Younger patients: HIV progression is also faster among patients who are younger than 13 years old, especially new born babies who are born with virus. This is likely because the newborn's immune is not yet fully developed. New born babies do not begin to make their own antibodies (proteins that detect the bind to foreign substances like viruses) until they are about six months old, (ARV therapy use in adolescents and adults, 2011).As people age, their bodies are not able to repair and rebuild damaged cells, organs or tissues as rapidly as those of younger people. Diseases like HIV that attack and destroy the body's defenses can exacerbate this slowing and increase the risk of developing additional medical problems like diabetes and high blood pressure, and more physical limitations than younger adults with HIV. In the early years of the HIV epidemic, (before HAART), adults' health deteriorated more rapidly than that of younger individuals, regardless of CD4 count. Several studies found that older adults had lower CD4 counts at diagnosis, faster progression to an AIDS diagnosis, more opportunistic infections, and a shorter survival rate than younger adults, regardless of when they were first diagnosed with HIV. Recent studies have found that a person's age does not interfere with the ability of HAART to reduce viral load, but there may be differences between younger and older people in how well the immune system responds to treatment.

A study published in *AIDS* (2000) by Roberto Manfred and Francesco Chiodo examined the effect of HAART on older people (defined as 55 or older) compared to younger people (35 or younger). The study included 21 older people (8 women, 13 men) and 84 younger people (29 women, 55 men). The researchers found that both groups responded to HAART, especially in reducing viral load. However, CD4 counts did not increase as much in the older people relative

to the younger ones. On average, CD4 counts increased from 212 to 289 for older adults after one year of HAART. During the same period, CD4 counts rose from 231 to 345 for younger people. Some people may have a very low CD4 count even though they have an undetectable viral load. This may be related to decreased activity in the thymus (the gland where CD4 cells are made). A 2001 study in *AIDS* conducted by researchers in Los Angeles included 80 HIV-positive veterans (13 were over 55 and 67 were younger). Although both groups of veterans showed dramatic reductions in viral load once they were on treatment, the researchers found significant differences in CD4 levels at 3, 9, 15, and 18 months. After one year on HAART, average CD4 counts increased by 50 for the older men, compared to increases of 100 for the younger ones. This difference was not related to baseline HIV viral load, co-infection with hepatitis C, or the race/ethnicity of participants. These studies represent an important first step in understanding how their age may affect older adults' response to HIV treatment, but more studies are needed to understand the long-term effects of age on HAART in older adults.

2. Nutrition

According to several studies, patients who have deficient levels of vitamin A, vitamin B12, or zinc are more likely to experience a rapid decline of cell counts. This is because the body’s white blood cells need sufficient levels of these vitamins in order to grow and maintain health. Researchers believe that poor absorption of nutrients, diarrhea, inadequate calories and protein consumption contribute to HIV progression. For instance, researchers found that poor nutrition in Zambia was the best predictor of the death in both HIV- negative and HIV- positive children. Several studies have shown that multivitamin supplementation can show the rate of HIV disease progression and death. However, HIV patients who have gastrointestinal problems may have a difficult time absorbing these essential vitamins into the bloodstream. Some HIV patients may need to take vitamins that are injected or in a form that will dissolve in the mouth and be absorbed across the mucus membranes. (Guide for HIV/AIDS clinical care, Jan 2011)

3. Location

It has been suggested that Africans living in the United Kingdom develop AIDS and die more quickly than non-Africans. However, recent research does not support this claim. According to a review of more than 1.050 HIV-infected Africans and 992 HIV- infected non-Africans diagnosed with the disease between 1982 and 1995, there was no a significant difference in survival rates between the two groups. The Africans lived an average of 82 months, while the non-Africans lived an average of 78 months. The researchers also reported no significant difference in the CD4 cell count or rates of progression. The researcher suggested that it was highly likely that the African patients studied were infected with a different strain of HIV (called HIV-2) than the one that normally infects homosexual men and injection-drug users in Europe and North America (called HIV-1). If HIV-2 does not cause HIV progression quicker outside of Africa, this suggests that environmental factors like lack of access to ARV therapy and treatment for opportunistic infections leads to the faster progression rates in Africans. Other researches suggested that there are no significant progression in Ugandans and the developed countries, (Guidelines on HIV therapy, 2006). However, some studies suggested that a common genetic mutation among Africans may increase the patient's risk of developing HIV, and the increase rate of disease progression. Other studies conducted in USA found no difference in viral load levels among different racial groups after controlling access to medical care, socio-economic status and CD4 cell count. (Guidelines for HIV therapy, 2006)

4. Gender

Based on several studies in both adults and children, it appears that gender does not affect the risk of HIV disease progression. In general, women have a lower viral set- point than men. The viral set- point is the point at which HIV replication slows and is suppressed by the body's white blood cells. However, this lower set-point does not appear to influence the rate of HIV disease progression and CD4 increase. (Guidelines for ARV therapy use, 2011)

5. Other factors

Factors other than HIV and the ones cited above can affect your CD4 count including infections, time of day, alcohol, smoking, stress, recreational drug use, infection route, genetics, pregnancy, repeated exposure to HIV and experience of health care providers.

2.2.4. Previous studies

2.2.4.1. Study finds that CD4 counts are less likely to recover when antiretroviral therapy is started late

Results of a recent study show that people who start HIV treatment later, at low CD4 counts, are less likely to achieve and maintain healthy CD4 counts after starting antiretroviral therapy. The study also showed that most of the increase in CD4 count after starting antiretroviral therapy occurs within the first 3 years after starting treatment, although CD4 counts may continue to improve for up to 7 years after starting antiretroviral therapy. The inability to normalize CD4 counts among many patients starting antiretroviral therapy at low CD4 counts, even after 7 years of treatment, provides additional support to consider initiation of therapy at higher CD4 counts, said Professor Judith Lok, lead author on the study, in correspondence with The AIDS Beacon.

To evaluate the existing treatment-initiation guidelines, researchers in this study compared people who started HIV treatment at higher CD4 counts to those who started at low CD4 counts. In particular, they examined whether participants' CD4 counts increased over time once they began taking antiretroviral medications, and if so, how much.The study monitored 575 HIV-positive participants from the time they first started antiretroviral medications. Researchers recorded the participants' CD4 cell counts before beginning antiretroviral therapy and at 3 years and 7 years after starting treatment.

Results showed that after 3 years the median CD4 count among all study participants increased from 270 to 556 cells per microliter. After 7 years results were similar, with a slightly lower median CD4 count of 532 cells per microliter of blood. Results also showed that CD4 counts 7 years after starting antiretroviral therapy were highly dependent on initial CD4 counts. Study participants who started with CD4 counts above 500 cells per microliter had a final median CD4 count of 724 cells per microliter. Participants whose initial CD4 counts were less than 200 cells per microliter had a final median CD4 count of 453 cells per microliter.

Participants who started antiretroviral therapy at CD4 counts of 200 or less were also four times more likely to have CD4 counts under 350 or to have died at the end of the 7 year period than participants who started at CD4 counts of 500 or more. The researchers concluded that the CD4 counts of most patients using antiretrovirals increase for at least 7 years after treatment initiation. However, they also suggested that antiretroviral therapy is less effective at increasing long-term CD4 counts when it is begun at lower CD4 counts. The study, named Strategic Timing of Antiretroviral Treatment (START), is a joint effort by the University of Minnesota, the National Institutes of Health, various European HIV institutes, and several pharmaceutical companies. The study is currently recruiting participants. (By Caitlin McHugh and Courtney McQueen Published: Nov 3, 2010 3:29 pm)

2.2.4.2. Relationship between Total Lymphocyte count (TLC) and CD4 count among peoples living with HIV, Southern Ethiopia: a retrospective evaluation

CD4 count is a standard measure of immunodeficiency in adults infected with HIV to initiate and monitor highly active antiretroviral therapy; however, it may not be feasible in resource poor countries. There is a need to have another marker of immunodeficiency that is less resource demanding. The objective of this study was to assess the relationship between total lymphocyte count and CD4 count in one of the resource poor countries, Ethiopia.

This was a retrospective evaluation. A total of 2019 cases with total lymphocyte and CD4 counts from three hospitals (Yirgalem, Hossana and Arba-Minch) were included in the study. Pearson correlation, linear regression and Receiver Operating Characteristic (ROC) were used.For adults, the sensitivity, specificity, positive and negative predictive values of TLC < 1200 cells/mm^3 to predict CD4 count < 200 cells/mm^3 were 41%, 83.5%, 87.9% and 32.5%, respectively. For subjects aged less than 18 years, these values were 20.2%, 87%, 82% and 27.1%, respectively. A TLC ≤ 1780 cells/mm^3 was found to have maximal sensitivity (61%) and specificity (62%) for predicting a CD4 cell count of < 200 cells/mm^3. Meanwhile, a TLC ≤ 1885 cells/mm^3 would identify only 59% of patients with CD4 count of < 350 cells/mm^3 (sensitivity, 59%; and specificity, 61%). The combined sensitivity and specificity for patients above 40 years of age was greater. They concluded by saying that ′Our data revealed low sensitivity and specificity of TLC as a surrogate measure for CD4 count,(By Deresse Daka and Eskindir Loha**,** 2008).

1.2.5. Standard method of counting CD4

1.2.5.1. Flow cytometry

Flow cytometry (abbreviated: FCM) is a technique for counting and examining microscopic particles, such as cells and chromosomes, by suspending them in a stream of fluid and passing them by an electronic detection apparatus. It allows simultaneous multiparametric analysis of the physical and/or chemical characteristics of up to thousands of particles per second. Flow cytometry is routinely used in the diagnosis of health disorders, especially blood cancers, but has many other applications in both research and clinical practice. A common variation is to physically sort particles based on their properties, so as to purify populations of interest.

Principle

A beam of light (usually laser light) of a single wavelength is directed onto a hydrodynamically-focused stream of fluid. A number of detectors are aimed at the point where the stream passes through the light beam: one in line with the light beam (Forward Scatter or FSC) and several perpendicular to it (Side Scatter or SSC) and one or more fluorescent detectors. Each suspended particle from 0.2 to 150 micrometers passing through the beam scatters the ray, and fluorescent chemicals found in the particle or attached to the particle may be excited into emitting light at a longer wavelength than the light source. This combination of scattered and fluorescent light is picked up by the detectors, and, by analyzing fluctuations in brightness at each detector (one for each fluorescent emission peak), it is then possible to derive various types of information about the physical and chemical structure of each individual particle. FSC correlates with the cell volume and SSC depends on the inner complexity of the particle (i.e., shape of the nucleus, the amount and type of cytoplasmic granules or the membrane roughness). This is because the light is scattered off of the internal components of the cell. Some flow cytometers on the market have eliminated the need for fluorescence and use only light scatter for measurement of other flow cytometers form images of each cell's fluorescence, scattered light and transmitted light, (Lyamia et al 1996).

CHAPTER TWO: MATERIALS AND METHODS

This chapter discusses the methodology that was used while carrying out the research. It involves the study area, study design, study population, ethical consideration, sampling method, sample size, procedure and data collection.

2.1. Study area

This study has been done at Nyagatare District Hospital; eastern province, Rwanda

2.2. Study design

This is the prospective study which was conducted at Nyagatare Hospital and was completed within 2 months on 150 patients who attended the VCT service.

2.3. Study population

All HIV+ patients who attend VCT service in Nyagatare hospital

2.4. Sample size

The sample size was obtained using the following formula:

$$N = \frac{P \times Q}{(E/1.96)^2} = \frac{11 \times 89}{(5/1.96)^2} = 150, \text{ (Kato, 2004)}$$

Where:

N = the sample size

P = Highest prevalence ever recorded = 11

Q = 100 – P

E = Error = 5; constant = 1.96.

2.5. Sample strategy

Purposive sampling method was used because it allowed the collection of all sample sizes in a given period with a purpose in mind.

2.6. Data collection methods and procedures

Blood samples were collected from HIV+ patients who attended Nyagatare hospital in VCT service during the period of data collection after answering the questionnaire.

2.7. Instruments and Laboratory Test procedures

FACS count machine was used to count the CD4

- **Materials**

Total blood collected under EDTA tubes (tubes with anticoagulants)

Protecting materials (gloves, laboratory coats)

Biosecurity materials (biosafety waste plastic bags)

- **Reagents**

BD FACS count CD4 kit

BD FACS count control kit

BD multi-checked control

BD multi-checked control CD4 low

- **Equipment**

Electronic pipettes

Tips for the pipettes

Vortexer

Coring station

Tru COUNT tubes for the CD4 reaction

Working station of FACS count (tube holder)

- **Principle of FACS COUNT**

This assay is based on flow cytometry and is issued to enumerate CD3 and CD4 cells in the sample of peripheral venous blood obtained from an individual.

When whole human blood is put to the reagent tubes containing CD3 and CD4 antibodies labelled with fluorochrome, the reagents bind specifically to lymphocyte surface antigen. After an hour of incubation, a fixative solution is added to the reagent tubes, and the sample is subjected to the analysis in the instrument.

The sample passing through the flow cell comes in contact with their laser light, which causes the fluorochrome labelled cells to fluorescence. This fluorescence light provides the information necessary for the instrument to count the cells.

Method

- Mark the tube with laboratory number for each sample.
- Vortex the reagent tubes for 5 seconds set the vortexer at an average speed.
- Open the reagent tube with the coring station.
- Place the reagent tubes in the coning station.
- The lever is released back to its original position.
- Transfer the reagents tubes from coning station to the working station.
- Cover the working station to prevent light exposure.
- For each sample, pipette 50µl of the total blood in the corresponding tubes.
- Cover the tube and incubate them for 60 to 120 minutes at room temperature 20to 25 °c.
- Open the tube and add 50µl of fixative solution in each tube.
- Cover the tube.
- Put inside the machine for reading.

Procedures got from the manual of how to test CD4 established by (Ministry of health, National Reference Laboratory (2007), *CD4 testing using flow cytometry technics (FACS COUNT)*, Kigali.

2.8. Data analysis

Most of statistical methods were used by others on this study like X^2-test, cohort test and a Kaplan-Meier approach, SPSS but our study was analysed using excel which is more popular and mostly used by scientists and since we compared many variables and carrying out a survey.

CD4 results

After reading by the FACS count Machine, the results were printed out.

2.9. Quality control

Blind proficiency testing panels composed of low, medium and high CD4 counts was used.

2.10. Inclusion and exclusion criteria

All HIV+ patients were included in the study and those who fall in the period of data collection were used as study populations, patients who have attended VCT services for at least 1 year are the ones used. Exclusion criteria were the patients less than 1 year while in VCT.

2.11. Problems and limitations

2.11.1. Problems

a) Access to the internet was not easy

b) Lack of materials to use at the time of CD4 counting

c) Lack of the equipment since the hospital has one which for the patients so I had to wait and some times the machine had technical disturbances.

d) Recruiting and convincing the participants was some how not easy.

e) Shortage of reagents

2.11.2. Limitations

Lack of exact information like: when the patient came to know that he or she is HIV+.

2.12. Ethical considerations

All participants included in the study was informed and agreed to answer the questionnaire. The admnistration of Nyagatare Hospital were asked permission by the researcher and INES admnistration to implement the study and to use their materials and equipments. CODES were used on patients instead of names to secure them.

CHAPTER THREE: RESULTS PRESENTATION AND DISCUSSIONS

3.1 Results Presentation

Introduction

The population of the study were all those who started the ARV with a lower CD4 baseline of 350cells/µl and patients were expected to have a maximum of one year while taking ARV.Studies done, most of them used one year or three years but for us we considered one year because the effectiveness of the ARV starts after one year of ARV therapy(Pat.R et al,1999),The CD4 count were assessed basing on the first CD4 count made after six months of ARV taking and those with no CD4 count between six and twelve months were excluded. We had a cut-off of 55cell/µl (from Hermann.b, 2002) and people who had the increase of under 55cells/µl were said to be with a low count. Also a study done by Moree et al. (2001),where they were comparing the mean values of males and females in the increase of CD4 count after ARV taking, had a cut-off mean value of above 80.

Table 1: A table which shows the low and high CD4 counted and their percentages basing on the cut-off.

According to our study, the total number of people who did not increase their CD4 basing on the cut-off are 20 out of 150 which gives a percentage of 13.3 % and 130 increased their CD4 which gives a percentage of 86.7%. The following are the results:

Characteristic		Number Low CD4 count	Percentage of low CD4 count	Number of CD4 count increased	Percentage CD4 count increased
Age	Younger	4	2.6%	40	90%
	Older	16	10.6%	90	84%
Gender	Female	7	4.6%	68	90%
	Male	13	8.6%	62	82%
Location	Town	9	6%	58	86%
	Village	11	7.3%	72	86%

The above table shows how CD4 were gained according to the cut-off. In brief, the whole sample size gained their CD4 after ARV therapy but there are some gained and others who did not gain after comparing their results with the cut-off of 50cells/ml of blood. According to the table, the young are the ones with the least number of people who did not gain their CD4 and the old are the one with the highest value of not increasing their CD4 counts and this is discussed in our result discussion in order to know why old people do not increase their values compared to the others.

Table 2: a table which shows the mean of CD4 counts before ARV therapy according to age.

The table 2 below showed how the mean of CD4 counted before ARV therapy taking varied basing on age (old and younger)

		Mean	Maximum	Minimum
AGE	Old	320	1184	26
	Youth	343	1420	40

Table 2 showed the mean of the CD4 counted in youths and old people before taking ARV therapy. The mean difference of the two was 23 which was not big and significant in order to determine that which gender (male or female) had a chance of increasing the CD4 counts than another after taking the ARV therapy.

Table 3: a table which shows the mean of CD4 count after ARV therapy according to age

The following table shows how the mean CD4 counted after taking ARV therapy varied according to age as a factor.

			Mean	Maximum CD4 counted	Minimum CD4 counted
AGE	Old	CD4	569	2551	96
	Youth	CD4	706	2463	104

Table 3 shows how the mean average of CD4 was after taking ARV therapy. According to the table, youth had 706 mean while old had 567 mean and also the table shows the CD4 values gained where the highest value gained in youth was 2551 while 2463 in old and it shows that the lowest value gained in young is 104 and 96 in old. The mean difference between young and old is 137 which was the value above the cut-off mean of 100.

Table 4: a table which shows the mean of CD4 counts before ARV therapy according to gender.

This table below showed how the mean of CD4 counted before ARV therapy taking varied basing on gender

		Mean	Maximum CD4 counted	Minimum CD4 counted
GENDER	F	317	950	46
	M	337	1420	26

Table 4 showed the mean of the CD4 counted in urban people and in rural people before taking ARV therapy. The mean difference of the two was 20 which was not big and significant in order to determine that which gender (male or female) had a chance of increasing the CD4 counts than another after taking the ARV therapy.

Table 5: **a table which shows the mean of CD4 counts after ARV therapy according to gender**

This table showed us how gender affects the effect of ARV therapy after taking them by comparing the mean of males and females.

		Mean	Maximum	Minimum
GENDER	F	634	2551	104
	M	615	2463	96

According to the table 5, there is no significant difference between the mean of males and females. Male had 615 while females had 634 which had a difference of 19 which is a small difference and hence had no significant difference in taking ARV therapy in males and females.

Table 6: a table which shows the mean of CD4 counts after ARV therapy according to location

This table below showed how the mean of CD4 counted after ARV therapy taking varied basing on the location of residence that is people living in Nyagatare town(considered as urban) and people lived in Nyagatare villages (considered as rural).

		Mean	Maximum	Minimum
LOCATION	Town	645	2551	104
	Village	620	1600	96

Table 6 showed that no significant difference in mean of the CD4 counted in urban people and in rural people. The mean difference of the two was 25 which was not big and significant in order to determine that which gender had a chance of increasing the CD4 counts than another.

Table 7: a table which shows the mean of CD4 counts before ARV therapy according to location.

This table below showed how the mean of CD4 counted before ARV therapy taking varied basing on the location of residence that is people living in Nyagatare town(considered as urban) and people lived in Nyagatare villages (considered as rural).

		Mean	Maximum CD4 counted	Minimum CD4 counted
LOCATION	Town	328	1420	46
	Village	326	1184	26

Table 7 showed the mean of the CD4 counted in urban people and in rural people before taking ARV therapy. The mean difference of the two was 2 which was not big and significant in order to determine that which group (town or village) had a chance of increasing the CD4 counts than another after taking the ARV therapy.

Table 8: A table which shows results of CD4 counted according to variables which are age, gender and location.

This table summarises what was presented in the above table.

Characteristic		Low CD4(before ARV)	High CD4(after ARV)
Age	Older	320	569
	Younger	343	706
Gender	Female	317	634
	Males	337	615
Location	Town	328	645
	Village	326	620

The CD4 before taking ARV therapy were termed as low CD4 count and the CD4 after taking ARV therapy were termed as the high CD4.In general, the mean within a group(males, females, young, old, town and villagers them selves) increased and it increased significantly which means that ARV therapy have a positive effect on the increase of CD4 counts but between groups(that

is between male and females, between young and old and between urban and rural), there was no big difference between variables, e.g. there was no big difference between male and females, there was no a big difference between town and villagers but there was a mean difference between the old people and the young ones.

3.2: Discussions of results

In our study, we were focusing on factors of patients which are: the age, gender and location of residence. The following are the discussions made:

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GENDER

In this study there was no effect of gender on the CD4 count response to ARV therapy. Our results are similar to studies by Parsons et al. (2011), where he found no significance difference of gender in response to increase in CD4 count. They used a population of 3500 and had a p value of 0.08 while the statistical p value was 0.05 and hence found no statistically significant in gender. However they are other studies that have shown a difference in CD4 count responses to ARV therapy according to gender. According to the study by Moree et al. (2001), 79% of males increased their CD4s and 82% of females increased their CD4s. A similar study was done by Prins et al. (1999) who found out that women increase their CD4s than males that is, females increased from 106 to 815 cells/µl while males increased their CD4 from 223 to 727 cell/µl.

AGE

In this study there was a significantly higher CD4 count response to ARV therapy in young patients compared to old patients. In addition, there was a significant negative correlation between age of the patients and CD4 count response to ARV therapy. Our results are similar to results obtained by Elizabeth et al (2011).

Apparently old people do not increase their CD4s rapidly due to the aging of their immune system while younger people increase their CD4s rapidly due to their active and strong immune system which is able to recover rapidly and fight off infections. According to a meta-analysis of 38 studies that involved more than 13000 HIV patients, age and time were significant factors in determining the rate of HIV progression. Patients who developed HIV antibodies between the age of 15 and 24 lived an average of 12.5 additional years. These patients progressed to AIDS

after an average of 11 years. Patients who developed HIV antibodies between the age of 45 and 54 lived an average of 7.9 additional years. These patients progressed to AIDS after an average of 7.7 years. (Elizabeth et al, 2011).

Roberto et al (2000) studied CD4 count in relation to HAART and they found that older adults had lower CD4 count and faster progression to AIDS and opportunistic infections and a shorter survival rate regardless of when they were first diagnosed. They compared younger people 35 or younger and older people above 35 or older where in their study, 21 were older (8 women and 13 men) and 84 younger people (29 women and 55 men). They found out that these people respond to HAART especially in reducing viral load and they found out that CD4 count did not increase as much in older people relative to younger ones. On average, CD4 increased from 212 to 289 in older people after 1 year of HAART and during the same period CD4 increased from 231 to 345 for younger people. The reason for this difference they said was that; the older people have an old thymus (a gland which produces CD4) and hence less production. The same study was also done in Los Angeles in 2001 on AIDS where 80 HIV^+ veterans were used and found out that older people increased their CD4 counts by 50% while younger people increased their CD4 counts by 90 %.

LOCATION

Our results show that location of residence of patients did not significantly affect the CD4 count response of patients attending voluntary counselling services at Nyagatare Hospital. Other studies have found different results. They found out that people in the village do not go for their serology check up and by the time they go for their check up, they find out that they are serologically positive and they have reached so AIDS phase. McHugh and McQueen (2010) found that those patients who start late on ARV treatment have a slower CD4 count increase than those that start early. A study done on 4800 HIV+ people by Susan et al. (2001) on how CD4 increases with people living in urban areas and in rural areas found that 38% of rural people received good care and took the ARVs according to prescribed schedules while 73% of urban people received good care and took ARVs as prescribed. They concluded that care and good taking of ARVs in urban people makes them to increase their CD4 count than the people in rural areas. There was no difference in the CD4 count response to ARV therapy between the urban and rural patients of Nyagatare, suggesting care levels and the taking of ARV drugs was similar

between urban and rural locations In our study, town had an average mean of 645 and village had an average mean of 620 and the difference between the two was 25 and basing on Moree et al. (2001), this had no significant difference and the reason given by Susan et al. (2001), of good care taking and access to the treatment was not a factor to Nyagatare patients because the hospital has the doctors who are responsible for out reach and follow up of these patients and see whether they are taking the precautions well so from here, we concluded by saying that location did not affect the increase of CD4 count in response to ARV therapy in patients who attend VCT service of Nyagatare hospital.

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CHAPTER FOUR: CONCLUSIONS AND RECOMMENDATIONS

4.1 Conclusion

AS a conclusion to our study, age is the only factor which affects the CD4 increase in response to the ARV therapy. In our study, we used the mean value where age had a significant difference in mean where the young had a mean of 706 and the old had 569 mean value which had a significant difference. This conclusion was concluded by Elizabeth et al (2011), who was comparing the average mean of living (life span of HIV^+ people) in old and young and found out that the young live longer than the old after taking ARV therapy where the younger ones lived an average mean of 12.5 years while the old lived an average mean of 7.9 after taking ARV therapy and from here, we concluded that age do affect the increase of CD4 counts where the young ones increased more than the old ones.

In our study, we concluded that gender do not affect the increase of CD4 counts. This goes directly to our literature review where we came to see that gender do not affect the increase of CD4 count, (Guidelines for ARV therapy use, 2011), and also a study done by Parsons et al. (2011) , found no significant difference in males and females and concluded by saying that this was not a big difference in order to say that females do increase than males and in our study, females had an average mean of 634 and males had 615 which had no significant difference so we concluded by saying that gender do not affect the increase of CD4 after taking ARV therapy.

We concluded also by saying that location do not affect the increase of CD4 counts in patients who take ARV therapy. The study done by Julia et al, (2011), found no significant difference in location. They found out that village increased 58% while town increased 60% after taking ARV therapy which had no a significant difference and in our study, town increased 645 average mean and village increased 580 average mean which had no big difference and we concluded that there is no increase in CD4 counts after ARV therapy basing on location. . In our study, town had an average mean of 645 and village had an average mean of 620 and the difference between the two was 25 and basing on Moree et al. (2001), this had no significant difference and the reason given by Susan et al. (2001), of good care taking and access to the treatment was not a factor to Nyagatare patients because the hospital has the doctors who are responsible for out reach and follow up of these patients and see whether they are taking the precautions well so from here, we

concluded by saying that location did not affect the increase of CD4 count in response to ARV therapy in patients who attend VCT service of Nyagatare hospital.

4.2 Recommendations

4.2.1. To the patients

Since age do affect the increase of CD4 counts in response to ARV therapy, there is a need to detect HIV infection early at young age so that ARV treatment is started at an early age, when there is a greater like hood of a significant CD4 count increase which translates into increased immunity and longtivity of life for HIV positive people.

4.2.2. To Nyagatare Hospital

The study found out that some drugs like: Tenofovir, Nevirapine, Diovir, Lamividine, Effavirenz (first generation) in combination with Kaletra (second generation) produced a higher CD4 count response (see the appendix of the drug in response to ARV), it is recommended that where first generation fails, the second generation must be administered immediately without waiting long.

4.2.3. To the researcher

Carry a survey on how nutrition affects the increase of CD4 while taking ARVs

Increase the sample size in order to have a detailed study.

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APPENDICES

Appendix I: Definition of key terms

1. AN ANTIBODY: An antibody, also known as an immunoglobulin, is a large Y-shaped protein used by the immune system to identify and neutralize foreign objects such as bacteria and viruses.

2. CD4: A type of T cell involved in protecting against viral, fungal, and protozoal infections. These cells normally orchestrate the immune response, signaling other cells in the immune system to perform their special functions

3. HOMOSEXUAL: is romantic and/or sexual attraction or behavior between members of the same sex or gender. As a sexual orientation, homosexuality refers to "an enduring pattern of or disposition to experience sexual, affectional, or romantic attractions" primarily or exclusively to people of the same sex; "it also refers to an individual's sense of personal and social identity based on those attractions, behaviors expressing them, and membership in a community of others who share them.

4. IMMUNODEFICIENCY: a condition in which the ability of the immune system to produce antibodies is impaired

5. IMMUNITY: is a biological term that describes a state of having sufficient biological defenses to avoid infection, disease, or other unwanted biological invasion. Immunity involves both specific and non-specific components. The non-specific components act either as barriers or as eliminators of wide range of pathogens irrespective of antigenic specificity. Other components of the immune system adapt themselves to each new disease encountered and are able to generate pathogen-specific immunity.

6. LYMPHOCYTE: is a type of white blood cell in the vertebrate immune system

7. OPPORTUNISTIC INFECTION: is an infection caused by pathogens, particularly opportunistic pathogens (bacterial, viral, fungal or protozoan) that usually do not cause disease in a healthy host, i.e. one with a healthy immune system. A compromised immune system, however, presents an "opportunity" for the pathogen to infect.

8. PANDEMIC: is an epidemic(new cases of a certain disease, in a given human population, and during a given period, substantially exceed what is expected based on recent experience) of

infectious disease that is spreading through human populations across a large region; for instance multiple continents, or even worldwide.

9. T-CELLS: T cells or T lymphocytes belong to a group of white blood cells known as lymphocytes, and play a central role in cell-mediated immunity. They can be distinguished from other lymphocyte types, such as B cells and natural killer cells (NK cells) by the presence of a special receptor on their cell surface called T cell receptors (TCR). The abbreviation T, in T cell, stands for thymus, since this is the principal organ responsible for the T cell's maturation. Several different subsets of T cells have been discovered, each with a distinct function

10. T HELPER CELLS: (also known as **T_h cells**) are a sub-group of lymphocytes, a type of white blood cell, that play an important role in establishing and maximizing the capabilities of the immune system. These cells are unusual in that they have no cytotoxic or phagocytic activity; they cannot kill infected host cells (also known as somatic cells) or pathogens, and without other immune cells they would usually be considered useless against an infection. T_h cells are involved in activating and directing other immune cells, and are particularly important in the immune system. They are essential in determining B cell antibody class switching, in the activation and growth of cytotoxic T cells, and in maximizing bactericidal activity of phagocytes such as macrophages. It is this diversity in function and their role in influencing other cells that gives T helper cells their name.

Appendix II: Profile of the researcher and the institute

a) Curriculum Vitae (CV) of the researcher

Profile of the researcher

Surname: Tumwebaze	Date of birth: 24/06/1985
First name: Edith	Place of birth: Masaka-Uganda
Family name: Gikundiro	Nationality: Rwandese
Sex: Female	Mother's names: Mukantwali Agnes
Father's names: pastor Mugabo Eliah	Marital status: Single

Academic profile

2010-2011: A student at INES-Ruhengeri in Biotechnology

2005-2007: A student at Kigali Health Institute in Medical Laboratory Sciences

2001-2003:A'level studies at Gahini secondary school in Biology and chemistry option

1998-2000:O'level studies at Nyakayaga senior secondary school

1995-1997: primary studies in Rwanda at Nyakayaga primary school

1992-1994: Primary studies in Uganda at Malongo primary school

1990-1991: Nursery studies in Uganda at Malongo primary school

Working experience

2011 Middle up to date: A supervisor of National Reference laboratory at Nyagatare hospital and Gahini Hospital

2010-middle 2011: A laboratory technician at the University Teaching Hospital of Butare

2008-2009: A laboratory technician at Umutara polytechnic University

2004: A teacher at Cyabusheshe primary school

Other Qualifications

I hold a certificate of computer literate in Microsoft word, excel and power point.

Certificate of attendance at Makerere University in a training of 2 months in laboratory

Languages spoken

English

French

Kinyarwanda/Luganda/Lunyankole

Hobbies

Making friends

Order

Singing and listening to the music

I like swimming

A big spectator of football

b) Profile of the institute

INES is the Institute of Higher Education established on 17th November 2003 and the foundation stone was laid on 30th June 2003 by the president of the republic of Rwanda, His Excellency PAUL KAGAME.

Vision of INES

Universality in each individual; knowledge in order to unite and better serve the world.

Mission of INES

"To contribute, through the interactive conjunction between civil society, private sectors and public sectors, to national and regional development, by providing specialized higher education enhanced by research, in order to create competitive enterprises and well-paid employments".

Management team of INES

The team is made up of the following elements;

a) Academic team

Academic team is set out again in 5 faculties of INES

1. Faculty of Fundamentals and Applied Sciences
2. Faculty of Economics
3. Faculty of Social Sciences and Management
4. Faculty of Languages and Applied Linguistics
5. Faculty of Law

b) Administrative team

Rector: Fr. Dr. Deogratias Niyibizi

Vice-Rector in charge of academics and research:
Rev. Fr. Dr. Fabien Hagenimana

Vice-Rector in charge of Finance and administration:
Mr. Gaspard Twagiramungu

Appendix III: Questionnaire

Instructions

Fill in and answer the following questions

1. Participant Code…….....
2. Date of enrolment in the VCT service (day/month/year)
3. …….....
4. Gender…….....
5. Date of birth (day/month/year).....
6. Weight in kgs…….....
7. Residence
    a) Mudugudu…….....
    b) Cell.....
    c) Sector.....
8. Occupation…….....
9. When did you go for your serology check up …….....
10. How long did you take to commence ARV therapy after knowing that you are HIV+…….....
11. When did you start your ARV therapy…….....
12. When you started which drugs were you taking…….....
13. Did you change the drugs you started (Yes/No), if yes when did you change the drugs that you were taking.....?
14. If you changed the drugs what caused you to change.....

15. Are there any side effects of the drug you are taking now Yes/No
16. If any, cite them……...
    ……..
    …….....
17. What was your CD4 when starting ARV therapy…….....
18. What is your present CD4 values…….....
19. Is there any change on your health after starting ARV therapy…….....
20. What are those changes.....
    ……..
    ……..
21. Did you drink alcoholic beverages /tobacco before you started this therapy (Yes/No

Appendix IV: Project organization

The following people were assigned by different tasks in the research:

1. **Researcher:** perceived the idea of carrying out the study, coordinated and carried out all activities of the research

2. **Supervisor:** directed and supervised all the activities which concerned the research

3. **co-supervisor**: assisted the supervisor in all activities of the research

4. **Head of the department**: carried out administrative roles in facilitating the getting of the permission to carry out the study at Nyagatare hospital.

5. **INES:** provided ethical permission to carry out the study

Appendix V: List of drugs taken by HIV+ patients at Nyagatare Hospital in VCT service and their mean value

ARV	Mean
3TC-D4T-NVP	318.57
ABC-3TC-NVP	265.00
AZT-3TC-EFV	394.00
AZT-3TC-KALETRA	655.00
AZT-3TC-NVP	302.05
AZT-DAT-KALETRA	695.00
AZT-DDI-KALETRA	178.00
AZT-EFV	56.00
BACTRIM	82.50
COMBIVIR-EFV	112.00
COMBVIR	490.00
COVIR	55.00
D4T+3TC+NVP	397.25
D4T-3TC-EFV	287.67
D4T-3TC-NVP	234.18
DOUVIR	130.00
DUOVIR	239.33
DUOVIR-EFV	323.00
EFV-D4T-3TC	.00
EFVV-D4T-3TC	46.00
LAMIVUDINE	248.50
NEV-EFV	.00
NVP	363.25
NVP-TENOFOVIR	459.00
TDF	-406.00
TDF-3TC-AZT	125.00

FROM: TUMWEBAZE EDITH

TO: THE DIRECTOR OF NYAGATARE HOSPITAL

RE: APPLICATION FOR THE PERMISSION TO CARRY OUT THE RESEARCH

Dear Sir

I write to submit my application letter to your office to seek a permission to carry out my research at your hospital.

I am a final year student at INES –Ruhengeri in the Department of Biotechnologies, Faculty of Applied Fundamental Sciences. My research project is entitled ‘’ the effect of age, gender and location of residence on CD4 count response to ARV therapy in patients who attend Nyagatare Hospital voluntary counseling and testing service”.

Your positive response towards my request is highly appreciated.

Yours faithfully

TUMWEBAZE Edith