INSTITUT D'ENSEIGNEMENT SUPÉRIEUR DE RUHENGERI

Accredited by Ministerial Order N° 005/2010/Mineduc of 16 June 2010

FACULTY OF APPLIED FUNDAMENTAL SCIENCES

DEPARTMENT OF BIOTECHNOLOGIES

Scientia et Lux

COMPARATIVE STUDY OF WHITE BLOOD CELL LEVEL IN HIV POSITIVE PATIENTS UNDER ARVs AND THOSE WITHOUT ARVs

"Case of Gisenyi District Hospital"

Dissertation submitted and presented in partial fulfillment of the requirements for the award of Bachelor's degree in Biotechnologies

By: Misbah GASHEGU

Supervisor: Pierre MUJYARUGAMBA (MSc)

Co-supervisor: Evariste BIZIMANA (BSc)

Musanze, August 2013

B.P. 155
Ruhengeri
Rwanda

T : +250 788 90 30 30
F : +250 788 90 30 32
W : www.ines.ac.rw
E : inesruhengeri@yahoo.fr

INSTITUT D'ENSEIGNEMENT SUPÉRIEUR DE RUHENGERI

Accredited by Ministerial Order N° 005/2010/Mineduc of 16 June 2010

FACULTY OF APPLIED FUNDAMENTAL SCIENCES

DEPARTMENT OF BIOTECHNOLOGIES

Scientia et Lux

COMPARATIVE STUDY OF WHITE BLOOD CELL LEVEL IN HIV POSITIVE PATIENTS UNDER ARVs AND THOSE WITHOUT ARVs

″Case of Gisenyi District Hospital″

A Dissertation submitted and presented in partial fulfillment of the requirements for the award of Bachelor's degree in Biotechnologies

By: Misbah GASHEGU

Supervisor: Pierre MUJYARUGAMBA (MSc)

Co- supervisor: Evariste BIZIMANA (BSc)

Musanze, August 2013

B.P. 155
Ruhengeri
Rwanda

T : +250 788 90 30 30
F : +250 788 90 30 32
W : www.ines.ac.rw
E : inesruhengeri@yahoo.fr

DEDICATION

To:

Our parents;

Our brothers and sisters;

Our friends and colleagues.

ACKNOWLEDGEMENTS

First of all, we are thankful to the Almighty God for his incomparable love, for giving us safe life, guidance and abundant blessing during our studies at large and during this research.

It is our great pleasure to thank the Government of Rwanda for its good policy and its tireless efforts to promote the institutions of higher learning; otherwise this research could not have been carried out.

We humbly present my deepest heartfelt gratitude to the Director of Gisenyi District Hospital for according me the opportunity to this research.

We would like to express our thanks to INES – Ruhengeri administration for their immeasurable assistance during our course of study.

We highly acknowledge and appreciate the assistance and guidance of our supervisors Pierre MUNYARUGAMBA and Evariste BIZIMANA, who kindly accepted to despite on this work and other tasks allocated to them.

Our heartfelt thanks go to academic staff of Biotechnologies from whom we got knowledge and skills that enabled us to obtain this current level.

We are grateful to the entire Laboratory staff at Gisenyi District Hospital, and special thanks go to the head of laboratory Rehema NYIRAMUSABWA, the staff of Hematology and CD4 sections for their valuable time, vast knowledge and commitment to work which made my work enjoyable and successful.

In particular, we recognize generosity, wisdom, advice and support from parents, brothers and sisters during our studies and deployed efforts from all our family members.

May God bless you.

Misbah GASHEGU

ABSTRACT

Leucopenia has a serious impact on the quality of live for AIDS patients. Among patients with HIV/ AIDS, leucopenia is statistically a significant predictor of progression to AIDS and is independently associated with an increased risk of death. HIV disease progression is about 5 times more common in people with leucopenia. Rate of serious leucopenia has dropped considerably since people started using ARV in HIV treatment although almost half of people living with HIV still have mild to severe leucopenia.

The aim of this study was to compare white blood cell and their differential levels in HIV positive patients. White blood cells level could decrease in HIV positive patients under ARVs and those without ARVs. The differential could vary in both HIV patients started ARV and others not yet starting. HIV positive patients under ARVs could have lower CD4 cell level than those without ARVs.

A normal and pathologic range of white blood cell was defined to classify leucopenia. Data was processed and analyzed by Microsoft word and Excel. Researcher conducted this study after getting permission and informed consent was used to explain the benefit of the study.

It was found that severe leucopenia was found at 11.2%. However mild and moderate 9.5% to 8.7% respectively and in all study population, 29.4% were leucopenic to 70.6% who had normal white blood cell levels and in patients under ARVs, leucopenia occurred at 19.8% while for the patients who did not receive ARVs was at 9.6%, this showed that the patients under ARV were more leucopenic (19.8%) than those without ARV (9.6%) and severe leucopenia was more predominant in patients under ARV 10.7% while in patients without ARVs 0.9%, this may be due to severe immunosuppression which was marked to side effect of ARVs.

From the analysis, there was a decrease of WBC levels according to the HIV/AIDS disease is progressing and shows that white blood cell and their differentials level except monocytes and basophils in patients under ARVs is lower than those without ARVs.

RÉSUMÉ

La leucopénie a un impact sérieux sur la qualité de personnes vivant avec la maladie du SIDA. Parmi les malades avec VIH/SIDA, la leucopénie est un prédicateur considérable de la progression aux SIDA statistiquement et est associé avec un risque indépendamment augmenté de mort. La VIH/SIDA est une maladie de progression est approximativement 5 fois plus commun dans les gens avec la leucopénie. Le taux de leucopénie sérieux est diminue considérablement depuis que les gens ont commencé à utiliser les ARVs dans le traitement du VIH bien que presque la moitie de gens qui vivent encore avec VIH ait doux à leucopénie sévère.

Le but a était comparer le niveau de la cellule de globule blanche et leur différentiel dans les malades séropositifs. Le niveau des cellules de globule blanc pourrait être diminué dans les malades séropositifs sous ARVs plus que ceux sans ARVs. La différentielle pourrait être variez dans les deux malades du VIH qui ont commencé ARV et autres qui n'ont pas commencé. Les malades séropositifs sous ARVs pourraient avoir le niveau cellulaire de CD4 inferieur que ceux sans ARVs.
Le niveau normal et pathologique de globules blancs étaient défini pour classer la leucopénie. Tous les données ont été traité analysé par Microsoft Word et Excelle. Le chercheur conduit cette étude après avoir obtenu autorisation et consentement bien renseigné pour expliquer d'avantage cette étude.

Il a été trouvé que la leucopénie sévère était de 11. 2%. Cependant doux et en modère 9. 5% à 8. 7% respectivement et dans toute la population de l'étude, 29. 4% étaient des leucopénies à 70. 6% qui avaient le niveau normal cellulaires de globules blanc et dans les malades sous ARVs, les leucopénies se sont produits à 19. 8% pendant que pour les malades qui n'ont pas reçu ARVs était à 9. 6%, cela a montré que les malades sous ARVs étaient plus de leucopénie (19. 8%) que ceux sans ARVs (9. 6%) et la leucopénie sévère était plus prédominante dans les malades sous ARVs 10. 7% pendant que dans les malades sans ARVs 0. 9%, ce peut être dû à immunosuppression sévère qui a été marqué pour se mettre l'effet d'ARVs.

De cette analyse, il y avait une baisse de niveaux de globule blanc comme la maladie du VIH/SIDA a progressé et montre que le niveau de globule banc et leur differentiel sont inferieur dans les malades sous ARVs par rapport aux malades sans ARVs avec l'exception des monocytes et basophiles.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND ACRONYMS

AIDS:	Acquired immunodefiency syndrome
ARVs:	Antiretroviral Drugs
ART:	Antiretroviral treatment
APCs:	Antigen-presenting cells
AV-HALTS:	Antiretroviral hyper activation limiting therapeutics
AZT:	Zidovudine (an antiretroviral drug)
BD:	Becton Dickinson
CD:	Cluster of differentiation on T helper lymphocytes
CEO:	Clinical executive officer
CMV:	Cytomegalovirus
CYT 107:	Cytheris 107
DHHS:	department of health and human services
EDTA:	Ethylene Diamine Tetra Acetic
EU:	European Union
GDHL:	Gisenyi District Hospital Laboratory
HAART:	High active antiretroviral therapy
HIV:	Human immune virus
INES-RUHENGERI:	Institut d'Enseignement Supérieur de RUHENGERI
QBC:	Quantitative blood Count
UNAIDS:	Union of Nations for fight against AIDS
WBC:	White blood cell
WHO:	World Health Organization
G-CSF:	Granulocyte-Colony-Stimulating Factor
GM-CSF:	Granulocyte-Macrophage-Colony- Stimulating Factor
MAC:	Mycobacterium avium complex
M-CSF:	Macrophage-Colony-Stimulating Factor
PGL:	Persistent generalized lymphadenopathy
SVC:	Simplified volumetric counting
US:	United State
2-SDS:	Two standard deviation

LIST OF TABLES

LIST OF FIGURE

LIST OF APPENDICES

GENERAL INTRODUCTION

1. Background

Hematologic parameters, white blood cells count (WBC) with their differential cell count, among others are important monitoring tools for assessing treatment and prognosis in HIV/AIDS. Apart from the CD4 count, a full blood count is the commonest pre-treatment investigation done for people living with HIV.

Although it is not part of the criteria for initiating therapy nor used by the World Health Organization (WHO) for staging HIV, hematologic abnormalities, indicated by a deranged full blood count, are common manifestations and important prognostic tools of human immunodeficiency virus (HIV) infection and AIDS. In all cases, a specific diagnosis of the cause, severity and mechanism of cytopenia should be sought, because a specific intervention other than the use of antiretroviral drugs may be indicated for its correction.

The use of antiretroviral drugs could positively or negatively affect these parameters, depending on the choice of combination used. Although many drugs used for the treatment of HIV-related disorders are myelo-suppressive, severe cytopenia is most often related to the use of zidovudine. Hence the need to review these parameters in a group of treatment-naïve HIV-infected patients only cannot be overemphasized (Akinbami *et al*, 2010).

HIV infection is mostly responsible for morbidity and mortality among adults and children especially in Sub-Saharan Africa; by the end of 2007, some 40 million persons worldwide were living with HIV or AIDS (UNAIDS 2007).

Whereas in 2005 an estimated 2-8 million lost their lives due to AIDS, in 2009, the number of people living with HIV in sub-Saharan Africa reached 22.5 million (20.9 million–24.2 million), 68% of the global total while the number of people living with HIV in a wide variety of hematological changes as a result of marrow defects and immune cytopenias directly resulting from HIV infection, opportunistic infections or lymphoma and the side-effects of drugs used to treat HIV itself or complicating infection or lymphoma.

The result of marrow defect associated singular targeting of individual hematological parameters usually leads to severe changes in the profile of these infected people. Example is medication-induced leucopenia, particularly from zidovudine (ZDV). A study by Dangana discovered that ESR results of HIV/AIDS subjects were increased significantly when compared to values of control subjects as a result of decreased leucocytes count that resulting in leucopenia. Additionally, HIV destruction of CD4+ T cells which regulate cellular and humoral immunity by interacting with other T lymphocytes, B lymphocytes, macrophages, and natural killer cells does results in a decrease in WBC counts with its associated increased infections in these patients.

HIV infection is accompanied by marked hematological changes that complicate health and treatment of patients. Hence it is important to determine the exact and extent of hematological changes in HIV patients which will lead to a holistic treatment and improve quality of life of these patients (Balt CA and Nixon H, 2001).

Leucopenia is observed frequently during HIV-1 infection. For example, evaluation of a large number of incarcerated adults revealed that leucopenia correlated strongly with HIV seroposi-tivity, independent of other variables, such as sex with an HIV-positive (HIV) partner, injection drug use, ethnicity, and presence of sexually transmitted diseases. (Stebbing *et al*, 2004)

Interestingly, in these studies, the risk for HIV seropositivity associated with leucopenia and a history of sex with an HIV partner were similarly high, underscoring that leucopenia is strongly associated with HIV positivity in at-risk persons and is commonly observed soon after HIV infection.

Of note, although the sinequanon feature of infection with HIV is progressive immunodeficiency related to CD4 T-cell lymphopenia, CD4 T-cell counts contribute minimally in quantitative terms to the overall white blood cell (WBC) count and their decline during the course HIV infection contributes minimally to this observed leukopenia, which is mainly attributable to neutropenia.

However, the impact of leucopenia on HIV disease course is largely undefined. Most of the studies that have examined the frequency or HIV disease-influencing effects of cytopenias have used cross-sectional study designs (eg, HIV vs HIV1 or HIV neutropenics vs HIV nonneutropenics). These studies have documented that the prevalence of cytopenias is higher in advanced disease (Florence *et al*, 2004)

Leucopenia is also encountered in patients with HIV. Although low leucocyte counts usually reflect the toxicity of therapies for HIV or associated conditions, studies of untreated patients have also shown a high incidence of leucopenia, particularly in patients with more profound immunodeficiency as a consequence of the virus (Doukas MA, 1992).

2. Problem statement

Leucopenia has a serious impact on the quality of live for AIDS patients. Among patients with HIV/ AIDS, leucopenia is statistically a significant predictor of progression to AIDS and is independently associated with an increased risk of death. HIV disease progression is about 5 times more common in people with leucopenia.

In Sub-Saharan Africa, for 70% of people with HIV /AIDS, the prevalence is higher than in developed countries and leucopenia is commonly caused by sickle cell disease, AIDS, malaria, hookworm infection and other infections (Mildvan D, 2003).

But no related studies have been done in Rwanda. The rate of serious leucopenia has dropped considerably since people started using ART, although almost half of people with HIV still have

mild to moderate leucopenia and several factors are linked to higher rate of leucopenia in people living with HIV: low CD4 cell count, high viral load, taking AZT, being African-American and being a woman (RS Ferri *et al*, 2001).

Blood transfusion is often the only treatment of severe leucopenia cases. However transfusion can cause infection by increasing opportunistic infections that appears to cause faster progression of HIV disease and increase the risk of death for HIV patient, alloimmunization and febrile non hemolytic transfusion reaction are common in patients receiving transfusion (Hoffman R *et al*, 2000).

In our study area, the HIV positive patients focused on ART only not on hematological changes while the variation of hematological parameters can cause the abnormalities of HIV patients such as: Leucopenia (Lymphopenia or Neutropenia).

3. Motivation

It has been observed that leucopenia is not early diagnosed for most of HIV positive patients; due to the pathology of leucopenia where the patients with HIV positive are at high risk to develop leucopenia which increase HIV disease progression and lead the high risk to the death; this research decide to evaluate that case by using WBC level as a biological criteria for leucopenia diagnostic. Although this the case, the research evidence indicates that such complication could be prevented through diet boosting and successfully treated when early was diagnosed.

4. Hypotheses

- White blood cells level could decrease more in HIV positive patients under ARVs than those without ARVs.
- The differential could vary in both patients started ARVs and those not yet starting ARVs.
- HIV positive patients under ARVs could have low CD4 level than those without ARVs.

5. Objectives

5.1 General Objective

To compare the blood cells level in HIV positive patients visiting Gisenyi District Hospital from June-July 2013.

5.2 Specific Objectives

- To compare white blood cells level in HIV positive patients under treatment and those not yet starting treatment.
- To compare the differential in HIV patients under ARVs and those without ARVs.
- To determine the immunosuppressant level based on CD4 cell count in HIV patients under treatment and those without treatment.

6. Significance of the study

6.1 Academic and scientific significance

This study would help INES-RUHENGERI students to understand more about leucopenia among HIV positive persons; it would be the basic guideline for other related studies and would found out if white blood cells with their differentials and CD4 cell counts reduction are related with severity of leucopenia.

6.2. Socio-economic significance

It is envisaged that the outcome of this study could be used by clinician's early detection to leucopenia in HIV infected persons. Further, the result of this study could be used to sensitize other medical personnel on the importance of leucopenia detection for patients with HIV.

This might in turn to decrease the complication of HIV such as: HIV disease progression, cost expenditure for both patients and government. For the patients: this may improve the quality of life by raising the awareness of the disease and help them to escape from what is likely cause or make the disease worse.

7. Methodology

7.1. Study area

This study was carried out in Gisenyi district Hospital located in Rubavu district, during one month.

7.2. Study population

This study was done on HIV positive patients under ARV and those without ARV; it includes all adolescents and adults (over 10 years) who were in monitoring for CD4 cell count.

7.2.1. Inclusion criteria

- Every patient who was under ARV and without ARV who were in screening for CD4 lymphocytes count.
- All HIV patients aged to 10 years and over
- Every patient who was monitored for CD4 and diagnosed hematological status at the same moment was also included.

7.2.2. Exclusions criteria

- The HIV positive patients who did not accept to participate and who were not monitored for CD4 lymphocytes counting.
- All HIV positive patients under 10 years.

7.3. Sample size

The sample size was 136 HIV positive patients under ARVs and those without ARVs who were in monitoring for CD4 lymphocytes counts and patients identities were used to know if patients was started to take ARVs or not.

7.4. Sample collection

Sample from HIV positive patients were collected in EDTA vacutainer tubes to allow hematological analysis and labeled with their proper code number, age, sex and specify if patient is under ARVs or not in order to separate specimen and data sheet was used in each group.

8. Subdivision of project

This work was subdivided into two main parts:

- Part I: Literature review

Chapter one describes function, structure and synthesis of WBC. It describe also the type of leucopenia, its classification and its biological diagnostic, classification of ARVs, the role of screening for CD4 counts in HIV positive patients, clinical stage of HIV, prevention and treatment of leucopenia.

- Part II: Experimental study

Chapter two describes the methodology used during data collection such as study area, study population with inclusion and exclusion criteria, sample size, sample collection, sampling strategy, data analysis, problem and limitation of the study.

In chapter three, the results are discussed and presented. The tables are used to illustrate the outcomes of the study.

Finally, chapter four comprises conclusion and recommendations.

PART I: LITERATURE REVIEW

CHAPTER I: GENERALITIES

Blood is certainly central to our survival, an organ we can't do without. It's the second most common tissue in the body (skin being the first), and comprises about 7% of a human's body weight (Battegay *et al*, 2006).

1.1.Production and function of blood cells

1.1.1. Production of blood cells

Blood cells are made in the bone marrow from stem cells. Blood passes through the bone marrow and picks up the fully developed blood cells for circulation in the blood.

1.1.1.1.Components of blood

- Erythrocytes (red blood cells)
- Leukocytes (white blood cells)

White blood cells (leukocytes) are an important component of the host defense system, responsible for protection against bacteria, fungi, viruses, and invading parasites. An intricate cytokine network and hierarchy of progenitor cells maintain baseline myelopoiesis and also allow rapid adjustment in the rates of production of these cell types that occur in response to acute and chronic stress (Amitic *et al*, 2008).

1.1.1.2.Structure and production of white blood cells

White blood cells originate from pluripotent haemopoietic stem cells.
Under the influence of various external stimuli (cytokines, matrix proteins, and accessory cells), stem cells develop into haemopoietic progenitor cells of various lineages. Growth factors that regulate the development of particular populations of white blood cells have been identified. For neutrophils, production involves several different growth factors, including granulocyte-colony-stimulating factor (G-CSF), granulocyte-macrophage-colony- stimulating factor (GM-CSF), interleukin 3, and macrophage-colony-stimulating factor (M-CSF).

In steady-state granulocyte production, G-CSF rather than GM-CSF is the most important lineage-specific factor according to findings in knockout mice. Similarly, for monocytes, both GM-CSF and M-CSF control number and function in vitro, but in mice only M-CSF deficiency leads to profound monocytopenia and macrophage deficiency (WHO, 2005).

- ✓ **Granular leukocytes:** have large granules in their cytoplasm and have bilobed nuclei.

❖ **Three kinds of granular leucocytes:**

- **Neutrophils:**

Pale lilac-first responders to foreign invasion, phagocytic, release enzymes. The largest pool of neutrophils is in the marrow (reserve pool), and a small number circulate in the peripheral blood (circulating pool); a number similar to that of the circulating pool exists in tissues (tissue pool).
The circulating pool can be further subdivided into two roughly equal compartments:

- ➢ A marginated pool of cells loosely adherent to vascular endothelium
- ➢ And a freely circulating pool.

Corticosteroids promote release of neutrophils from the marrow storage pool into the circulation and also inhibit movement of these cells from the blood into the tissues, resulting in an increased measured white-cell count. Intravascular activation of neutrophils (C5a, endotoxin) results in increased margination and hence a decrease in the measured neutrophil count.

- **Eosinophils:**

Red orange-phagocyte, release enzymes that combat the effects inflammation in allergic reactions, and effective against parasitic infections, Mature eosinophils, which make up 5–10% of granulocytes, have bilobed nucleus and numerous orange cytoplasmic granules. Eosinophils have substantial proinflammatory and cytotoxic activity and play an important part in the pathogenesis of various allergic, parasitic, and neoplastic disease processes.

- **Basophils**

Blue purple release substances that are involved in inflammation and allergic reactions, also called mast cells once in the tissue, basophils are the least common granulocytes and are distinguished from eosinophils by large metachromatic (purple-black) granules rich in histamine, serotonin, and leukotrienes. Mast cells are related to, but distinct from basophils.
Basophils are bilobed, whereas mast cells are long-lived cells that reside in tissues rather than peripheral blood and are capable of cell division. Both cell types are involved in immediate and cutaneous hypersensitivity reactions including asthma, urticaria, allergic rhinitis, and anaphylaxis (Balt CA and Nixon H, 2001).

- ✓ **Agranular leukocytes:** granules cannot be visible (so described as agranular)

- **Lymphocytes:**

B cell secretes antibody that is effective in destroying bacteria and deactivating toxins.

T cells attack viruses, fungi, transplanted tissue, and cancer cells. Lymphocytes represent about a third of the white blood cells in the peripheral blood and most have a compact rounded or gently notched nucleus with scant agranular cytoplasm. T cells are participating in cell-mediated immune responses. The remainders are B cells, which are programmed to produce antibodies.

B and T lymphocytes cannot be distinguished morphologically. Some 10% of lymphocytes are large granular lymphocytes, characterized by abundant cytoplasm and reddish granules.

Lymphocytes cells are called natural killer cells because of their ability to destroy virus- infected and HLA-incompatible target cells. 3–8% of circulating leucocytes are monocytes, which are characterized by their large size and folded nuclei.

After migration into extravascular tissues, they increase in size and acquire the morphological characteristics of tissue macrophages. Macrophages and monocytes play an important part in regulating the afferent and efferent components of the immune system.

- **Monocytes:** most important phagocytic cell. Called macrophage when enter the tissue.

✓ **Platelets (thrombocytes)** Are :

- Produced in the bone marrow
- membrane enclosed fragments of the megakaryocyte
- involved in blood clotting mechanisms
- life span is 5 to 9 days

✓ **Plasma**

➢ The liquid portion of blood:

Consist of 91.5% water and 8.5% solute (plasma proteins, gases, electrolytes, waste products, enzymes, hormones)

➢ plasma proteins contains:

- Albumin manufactured in the liver and is responsible for maintaining blood volume. Comprise 55% of the plasma proteins.
- Globulins Comprise 38% of the plasma proteins produced by the lymphocytes (antibodies) and functions in immunity.
- Fibrinogen- 7% of plasma proteins. Functions in blood clotting and is produced by the liver (Clark, 2009).

Figure which shows the different components of blood cell,

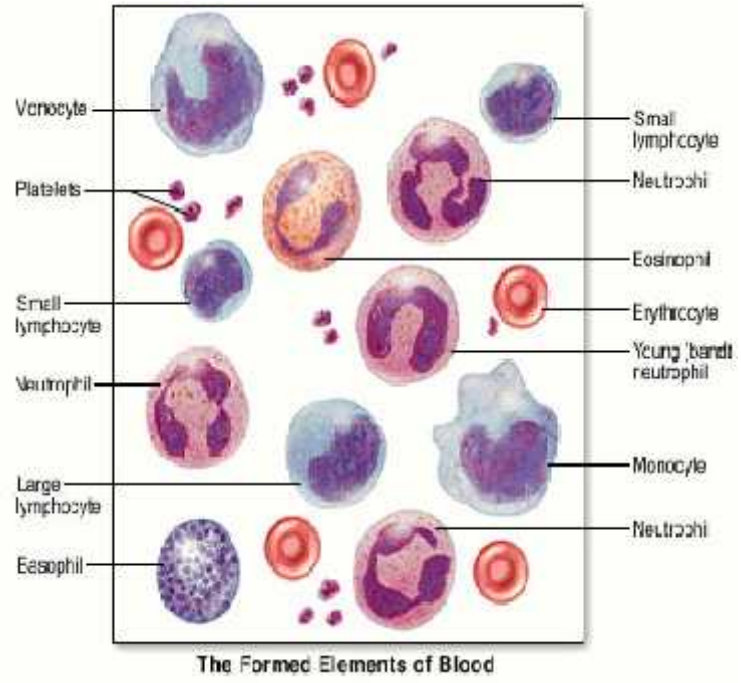


Figure 1: Components of blood

(Source: http//en.Wikipedia.org/components of blood cells, retrieved on 16th June 2013)

1.1.2. Functions of blood

- Transportation of oxygen, carbon dioxide, waste, nutrients and hormones
- Regulation of pH, temperature and influences water content of cells
- Protection of blood loss via clotting mechanisms and foreign microbes via white cells

Stem cells are multipotential cells that are capable of developing into different types of blood cells. Some stem cells enter the blood and circulate. Red blood cells carry oxygen from the lungs to cells throughout the body. Platelets are fragments of cells that help to control bleeding or bruising (Stebbing *et al,* 2004).

White blood cells include neutrophils, monocytes (macrophages), lymphocytes, eosinophils and basophils. Each plays a role in helping the body fight infection. For example, lymphocytes help create antibodies that attack the invading microbes and mark them for destruction by the neutrophils, monocytes and macrophages. Basophils and eosinophils are involved in the body's response to allergic reactions and eosinophils also help fight some parasitic infections.

White blood cells have involved in the adaptive immune response: antigen-presenting cells (APCs) not pathogen-specific ingest foreign substances and break them down macrophage dendritic cells. White blood cells are further grouped into monocytes (macrophages and dendritic cells), lymphocytes (T lymphocytes, B lymphocytes and Natural Killer cells) and granulocytes (neutrophils, basophils and eosinophils). T lymphocytes can be subdivided further into CD4+ Helper T lymphocytes and CD8+ Cytotoxic T lymphocytes. B cells and T lymphocytes (B or T cells) pathogen specific different types recognize different invaders and lead to their destruction. An absolute CD4 count is the number of CD4+ Helper T lymphocytes present in 1ml of blood. These cells carry the CD4 cell surface molecule (Cassens *et al*, 2004).

The CD4 percentage is expressed as the ratio of CD4+ Helper T lymphocytes to the total population of lymphocytes in blood. In children where lymphocytes are present in higher numbers, the absolute CD4 count is less reliable as a marker of HIV disease state and the CD4 percentage is preferred.

Lymph nodes are small structures that contain lymphocytes. Lymph vessels connect the lymph nodes. Peripheral lymph nodes are near the surface of the skin and can be felt by a doctor. Some examples of peripheral nodes are cervical (head and neck), axillary (the arm pits), inguinal (the groin) and popliteal (lower limbs).

Internal lymph nodes are inside the body and will show on imaging tests. Some examples of internal nodes are mediastinal (the area between the air sacs of the lungs), para-aortic (in front of the spine near the heart), iliac (the pelvic area) and inguinal (the groin).

Human blood contains many cell types of variable size and internal complexity. The three basic cell types are red blood cells (erythrocytes), white blood cells (leucocytes) and platelets (UNAIDS, 2003).

1.2. Leucopenia

Leucopenia is the decrease in the white blood cells concentration of the blood to levels below the normal range and is the most common blood abnormalities seen in people with HIV disease. White blood cells play a role in helping the body fight infection. One or more lineages may be affected in leucopenia, and there may be an imbalance in the white-cell subpopulations rather than a decrease in the absolute total count. One or more lineages may be affected in leucopenia, and there may be an imbalance in the white-cell subpopulations rather than a decrease in the absolute total count (Clark, 2009).

1.2.1. Classification of leucopenia

Leucopenia is observed frequently during HIV-1 infection. For example, evaluation of a large number of incarcerated adults revealed that leucopenia correlated strongly with HIV seropositivity, independent of other variables, such as sex with an HIV-positive (HIV) partner,

injection drug use, ethnicity, and presence of sexually transmitted diseases. Interestingly, in these studies, the risk for HIV seropositivity associated with leucopenia and a history of sex with an HIV partner were similarly high, underscoring that leucopenia is strongly associated with HIV positivity in at-risk persons and is commonly observed soon after HIV infection.

Of note, although the sinequanon feature of infection with HIV is progressive immunodeficiency related to CD4 T-cell lymphopenia, CD4 T-cell counts contribute minimally in quantitative terms to the overall white blood cell (WBC) count and their decline during the course HIV infection contributes minimally to this observed leucopenia, which is mainly attributable to neutropenia.

- **Neutropenia**

Neutropenia is defined as an absolute neutrophil count of more than 2 SDs below a normal mean value. Normal absolute neutrophil counts vary among ethnic groups; the lower limit of normal for white people is whereas black people have slightly lower counts, with a lower limit of normal of about. The lower absolute neutrophil count in black people has been attributed to a relative decrease in the size of the marrow storage pool. The degree of neutropenia predicts the risk of serious bacterial infections.

Only patients with severe neutropenia have an increased risk of developing life-threatening infections. Mild neutropenia may not predispose a patient to life- threatening infection, but should not be ignored since it may be the consequence of an underlying haematological disorder. The duration of the neutropenia commonly defines the risk to the patient. The acute onset of severe neutropenia is frequently associated with a high risk of infection and presents as fever and sepsis, whereas patients with severe neutropenia of more gradual onset may have far less threatening symptoms at presentation (Phillips AN and Lundgren JD 2006).

Severe neutropenia associated with fever of recent onset is a medical emergency requiring aggressive investigation and treatment. There are well recognized inherited forms and acquired forms of neutropenia. Some cases defy classification. Such patients are generally followed up for long periods before the underlying cause of their neutropenia becomes apparent. Neutropenia can, of course, be a manifestation of a more extensive marrow failure state such as aplastic anaemia, Fanconi's anaemia, myelodysplasia, or acute leukaemia. A large number of primary inherited haematological disorders present as neutropenia, predominantly during childhood. Congenital agranulocytosis (Kostmann's syndrome) is characterized by early onset of life-threatening bacterial infections, severe neutropenia, and maturation arrest of marrow granulopoiesis at the myelocyte/promyelocyte stage.

This disorder generally shows autosomal recessive inheritance, but sporadic and autosomal dominant patterns have been described. Affected children develop frequent and life-threatening

infections. Long-Severe neutropenia associated with fever of recent onset is a medical emergency requiring aggressive investigation and treatment. There are well-recognized inherited forms and acquired forms of neutropenia (panel 4). Some cases defy classification. Such patients are generally followed up for long periods before the underlying cause of their neutropenia becomes apparent. Neutropenia can, of course, be a manifestation of a more extensive marrow failure state such as aplastic anemia, Fanconi's anaemia, myelodysplasia, or acute leukemia. A large number of primary inherited hematological disorders present as neutropenia, predominantly during childhood. Congenital agranulocytosis (Kostmann's syndrome) is characterized by early onset of life-threatening bacterial infections, severe neutropenia, and maturation arrest of marrow granulopoiesis at the myelocyte/ promyelocyte stage.

This disorder generally shows autosomal recessive inheritance, but sporadic and autosomal dominant patterns have been described. Affected children develop frequent and life-threatening infections. Long- term G-CSF therapy is effective and well tolerated, and leads to a sustained neutrophil response (associated with a reduction in the rate of severe infections and the use of intravenous antibiotics) in 91% of cases. During long-term follow-up, 16 of 220 patients with congenital agranulocytosis maintained on chronic G-CSF therapy developed acute myeloid leukemia or a myelodysplastic disorder (Balt CA and Nixon H, 2001).

This transformation is thought to be a consequence of the intrinsic stem-cell defect that leads to the congenital agranulocytosis. Mutations of the receptor for G-CSF resulting in the truncation of the C-terminal maturation domain are associated with progression from congenital agranulocytosis to myelodysplasia or acute myeloid leukemia. For patients who do not respond to G-CSF, allogeneic stem-cell transplantation is the only curative approach.

Cyclical neutropenia is a rare disorder caused by a stem-cell regulatory defect; it is characterized by a transient severe neutropenia that occurs roughly every 21 days. The nadir neutrophil count lasts 3–7 days and is frequently associated with monocytosis. The nadir absolute neutrophil count is generally between zero and the marrow is characterized by transient arrest at the promyelocyte stage before each cycle. Cycling of platelets and red-cell production is also observed in some cases. The patients present with a history of recurrent fever, pharyngitis, stomatitis, and, in some cases, life-threatening bacterial infections

(Source: http//www.ncbi.nml.nih.gov/pubhem/12308654, retrieved on 29th June 2013).

Most patients present in childhood but there is also an adult-onset form of the disease. The familial form seems to be inherited in an autosomal dominant pattern. Genetic linkage analysis of affected families has allowed the locus for cyclical neutropenia to be mapped to chromosome where several different mis-sense and splicing mutations have been found in the gene encoding neutrophil elastase, a chymotryptic serine protease of neutrophils and monocyte granules.

G-CSF therapy of cyclical neutropenia is not associated with the development of acute myeloid leukemia or myelodysplasia. Schwachman-Diamond-Oski syndrome is an autosomal recessive syndrome characterized by the triad of neutropenia, metaphyseal dysplasia, and pancreatic insufficiency. The absolute neutrophil count is less than in most patients, and many are thrombocytopenic and anaemic. Other physical abnormalities, including short stature, cleft palate, and microcephaly, are common. Some patients eventually develop acute leukemia or aplastic anaemia, which suggests an underlying stem-cell defect.

The marrow is hypocellular at presentation in many patients. The gastrointestinal manifestations respond to pancreatic enzyme replacement and generally resolve by age 5–10years (http://scholar.google.com/scholar?q=Neutropenia+in+HIV+positive+patients%28pdf%29&btnG=&hl=en&as_sdt=0%2C5, retrieved on 05 June 2013).

G-CSF therapy has resulted in increases to normal of the absolute neutrophil count and is the treatment of choice in patients with recurrent infections. Stem-cell transplantation is reserved for patients who develop aplastic anemia or acute leukemia. Chediak-Higashi syndrome is a rare autosomal recessive disorder characterized by oculocutaneous albinism, progressive neurological abnormalities, and large blue-grey granules in the cytoplasm of neutrophils, eosinophils, basophils, and platelets. Patients develop severe neutropenia due to ineffective granulopoiesis and die, mostly in childhood, from infections involving the skin or pulmonary tract or an unusual lymphoproliferative disorder. Mutations in the lysosomal trafficking regulator, or LYST gene, located on chromosome 1q43, have been implicated as the cause of the syndrome. Mutations in LYST result in defective T-cell signalling, which may lead to the development of the associated lymphoproliferative syndrome seen in patients with Chediak-Higashi disease (Balt CA and Nixon H, 2001).

Reticular dysgenesis is characterized by agranulocytosis, lymphoid hypoplasia, and thymic dysplasia associated with normal erythropoiesis and thrombopoiesis. Patients have low serum concentrations of IgG and IgM and die from overwhelming bacterial and viral infections. Dyskeratosis congenita is a rare X-linked bone-marrow-failure syndrome characterised by abnormal skin pigmentation, nail dystrophy, and mucosal leucoplakia.

More than 80% of patients develop bone-marrow failure, which is the major cause of premature death. Hyperimmunoglobulin-M syndrome is an X-linked disorder characterised by lymphoid hyperplasia and low concentrations of IgA and IgG but low concentrations of IgM. Many of these patients develop severe neutropenia. This disorder has been attributed to a genetic defect in the T-cell CD40 ligand. Patients die of overwhelming infection by age 5 years unless they receive intravenous immunoglobulins and long term G-CSF therapy.

Most are due to dose-dependent marrow suppression or immunological mechanisms. The neutropenia generally develops within 1–2 weeks of the start of drug therapy. The most common offending agents are phenothiazines, non-steroidal anti-inflammatory agents, gold salts, ibuprofen, sulphonamides, antithyroid medications, anticonvulsants, high-dose semisynthetic penicillins, vancomycin, clindamycin, ganciclovir, and H2 blockers. If the neutropenia is mild or moderate (absolute neutrophil count and no substitute is available for the drug, continued administration with close observation is reasonable (WHO, 2005)

Viral infections postinfectious neutropenia commonly accompanies viral infections. Infection with HIV and the development of AIDS are associated with mild to moderate neutropenia. In children, varicella, measles, rubella, infectious mononucleosis, and influenza may result in neutropenia. Infections with hepatitis A and B viruses, parvovirus, and cytomegalovirus also result in neutropenia. Virus-induced neutropenia generally begins within a few days of the viral infection and persists for several weeks. This degree of neutropenia is most common in neonates and in debilitated adults, and is due to exhaustion of the neutrophil marrow reserve pool. It has a poor prognosis.

Autoimmune neutropenia, an isolated neutropenia, has been reported in patients with various autoimmune disorders, including systemic lupus erythematosus, rheumatoid arthritis, autoimmune thrombocytopenic purpura, and autoimmune haemolytic anemia. IgG or IgM antibodies may be directed against mature neutrophils or marrow precursors. Felty's syndrome is a well-described collection of clinical abnormalities consisting of rheumatoid arthritis, splenomegaly, and severe neutropenia. The cause has been attributed to increased neutrophil margination and inhibition of granulopoiesis mediated by antibodies to neutrophils or by T cells.

Although many patients with this disorder have no symptoms, proportions have serious recurrent bacterial infections. Many such patients show great improvement after G-CSF therapy during an acute infectious process and require long-term therapy. Lymphoproliferative disease of granular (T) lymphocytes commonly manifests as severe neutropenia with a median age at onset of 55 years.

It results from the clonal proliferation of either CD3-positive (T) or CD3-negative (natural killer) cells. The natural killer cells do not have rearranged T-cell-receptor genes, whereas the CD3-positive large granular lymphocytes have clonally rearranged T-cell-receptor genes and are thought to represent in-vivo-activated cytotoxic T lymphocytes. The disease is characterized by recurrent bacterial infections that occur as a consequence of severe neutropenia.

Some patients with adult-onset cyclical neutropenia also have this disorder. The finding of more than 2·010 /L large granular lymphocytes persisting for more than 6 months is required to confirm the diagnosis. There is diffuse lymphocytic infiltration of the marrow with maturation arrest of the

myeloid cells. The expansion of the large granular lymphocyte population can be established by use of flow cytometry (Richard *et al*, 2001).

Since the CD3-positive CD16-positive cell population accounts for less than 5% of normal cells, an increase in this cell population is characteristic. For the CD3-positive form, rearrangement of T-cell-receptor genes can be detected by Southern blotting, which confirms the clonal origin of the disorder. CD3-positive lymphoproliferative disease of granular lymphocytes generally has an indolent, but not altogether benign course. Most patients need treatment for the recurrent episodes of life-threatening infection. G-CSF therapy is effective in treating acute infections, and methotrexate, prednisone, cyclo- phosphamide, or cyclosporin result in improvement in neutrophil counts for sustained periods.

In contrast, the CD3-negative CD56-positive (natural killer) disorders can be clinically aggressive; they tend to occur in younger patients. These patients present with fever, massive hepatosplenomegaly, and jaundice. Blood, bone marrow, and other organs are infiltrated with lymphomatous cells. This disorder typically has a rapidly progressive course and is refractory to combination chemotherapy.

Pure white-cell aplasia is an acquired disorder characterised by severe neutropenia or a total absence of neutrophils, due to an IgG or IgM antibody directed against granulocytic precursor or progenitor cells. Many patients with this disorder have a history of thymoma. The marrow has no myeloid precursor cells but shows normal erythroid and megakaryocyte development and a normal karyotype. If thymectomy is not effective, immuno-suppressive therapy, including steroids, intravenous immunoglobulins, cyclophosphamide, or cyclosporin may be effective. Isoimmune neonatal neutropenia, occurring in 0·5–2·0 per 1000 live births, develops antenatally and can be recognized at delivery or during the first days of life. This diagnosis should be considered in any infant with neutropenia that persists for several days. The disorder is due to the maternal production of IgG directed against antigens on the fetal neutrophils. Sensitisation to fetal antigens can occur at any time during gestation, and the IgG antibody is transferred to the fetus across the placenta.

Other cause of neutropenia has been associated with deficiencies of vitamin B12, folate, and copper. These disorders are characterized by ineffective granulopoiesis that is corrected by appropriate supplementation. Hypersplenism can lead to neutropenia, rarely severe enough to lead to symptoms; this neutropenia is frequently accompanied by anaemia or thrombocytopenia and the marrow is hypercellular with normal myeloid development. Complement activation leading to generation of C5a can lead to acute and chronic neutropenia (Akimbani *et al*, 2010).

- **Lymphopenia**

Severe combined immunodeficiency is a heterogeneous group of disorders characterized by profound deficiency of both T and B lymphocytes. These disorders may show autosomal recessive or X-linked inheritance. The X-linked form is the most common and is characterized by the absence of mature T cells and natural killer cells in the presence of normal numbers of poorly functioning B cells. The molecular defect in this form is a mutation in the chain that is common to the receptors for interleukins 2, 4, 7, 9, and 15 and accounts for 50–60% of cases of severe combined immunodeficiency (Mayo, 2009).

A deficiency of adenosine deaminase underlies 15% of cases in total, and 30–40% of cases of the autosomal recessive form. Adenosine deaminase deficiency leads to accumulation of the toxic metabolites of purine metabolism, deoxyadenosine triphosphate and 2- deoxyadenosine.

The different forms of severe combined immunodeficiency are clinically indistinguishable, and present in early infancy with severe recurrent infections due to opportunistic viral and fungal infections resulting in failure to thrive. The diagnosis is a medical emergency, since correction of the defect by stem-cell transplantation or enzyme replacement with adenosine deaminase treated with polyethylene glycol leads to clinical improvement or cure. Transfer of the common chain into CD34-positive cells of two children with severe combined immunodeficiency has been reported (Odani S *et al*, 2005).

1.2.2. HIV/AIDS and leucopenia

Leucopenia is the most common complication observed in people with human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS). Up to 96% of HIV/AIDS patients may have leucopenia. Key factors: In HIV/AIDS patients, all three types of blood cells are frequently reduced: Leucopenia (white blood cells reduced), Anemia (red blood cells reduced) and Thrombocytopenia (platelet reduced).

Leucopenia in HIV patient is multifactorial: HIV infection itself influences all haemopoietic cell lineages, and results in serious hematological abnormalities. Beside leucopenia, anemia (red blood cells reduced) and thrombocytopenia (platelet reduced) are all common during the course of disease. Inflammatory cytokines generates negative effects on leucopoietin levels and white blood cell production, complications of HIV includes kidney and bone disorders. These organs are critical for health blood production (.

Thus, these complications may induce leucopenia, or make it worse. In addition drug toxicities, opportunistic infection, malabsorption syndromes leading to foliate or vitamin B12 deficiency,

blood loss and parvovirus B19 infection can all cause leucopenia AIDS treatment has demonstrated its unique advantages: remarkable improvement not only in general symptoms, but also in lab findings including virus loading and CD4 T lymphocyte count improve the symptoms and signs of AIDS, enhance the immune function, decrease the possibility of contracting opportunistic infection, improve the life quality, prolong the survival period with no apparent toxic and side effects (Balt CA and Nixon H, 2001).

1.2.3. Impact of leucopenia on HIV positive patients

However, the impact of leucopenia on HIV disease course is largely undefined. Most of the studies that have examined the frequency or HIV disease-influencing effects of cytopenias have used cross-sectional study designs (eg, HIV^+ vs HIV^- or HIV^+ neutropenics vs HIV^+ nonneutropenics). These studies have documented that the prevalence of cytopenias is higher in advanced disease. For example, neutropenia ranges from 0.8% to 13.4% when $CD4^+$ counts are less than 250cells/mm^3 and from 13% to 44% in those with AIDS. By contrast, there are no prospective studies in natural history cohorts that have examined whether leucopenia impacts on HIV disease course, independent of the known strong relationship between immunodeficiency (reflected by low CD4counts or high viral loads) and either leucopenia or neutropenia. To address this, we determined whether leucopenia impacts on the HIV disease course of subjects in a natural history cohort.

Neutropenia ranges in HIV patients based on CD4 count

Table 1: Ranges of neutropenia based on CD4 count

Neutropenia ranges (%)	**CD4 counts levels (cells/mm^3)**
0.8 - 13.4	<250
13 – 44	AIDS

(Source: http:// en.wikipedia.org/wiki/ Neutropenia, retrieved on 24th May 2013)

The large representation of both European Americans (EAs) and African Americans (AAs) in the study population allowed us to examine whether the impact of leukopenia on disease course differed according to race. This possibility is of particular interest because persons of African ancestry, on average, have significantly lower WBC counts, Secondary to lower neutrophil cell counts than persons of European descent. In otherwise healthy persons, low WBC counts in persons of African ancestry are thought to be both genetically determined and benign, as they have not been associated with an increased incidence of bacterial infection. This has resulted in the designation of this condition as “benign ethnic leukopenia or neutropenia”.

In light of the preceding discussion, we considered whether differences in WBC counts also result in racial differences in HIV disease outcome. Such an analysis could be confounded by social factors (eg, access to health care, socioeconomic status). However, our studies were conducted in a well-characterized natural history cohort of HIV infection in which several factors that may confound assessment of race-specific differences in disease progression were minimized (eg, equal access to health care, similar living standards, and minimal loss to follow-up).

Leucopenic range of white blood cells for human being:

Table 2: Leucopenic ranges of white blood cells:

Severity	**White blood cells Range (x 10^3/µL)**
Mild	4.0 - 3.5
Moderate	3.5 - 3.0
Severe	3.0- 2.5

(Source: http:// en.wikipedia.org/wiki/ Leucopenia, retrieved on 24th May 2013)

1.2.3. Diagnosing of leucopenia

Leucopenia occurs when a patient has a lower than normal amount of white blood cells especially lymphocytes and neutrophiles, in abnormal of white blood cells or in defects of proteins metabolism etc.

Leucopenia is assessed by measuring the amount of white blood cells. Complete blood count (CBC) may also be used, which provides levels of white blood cells such as lymphocytes, neutrophiles and other hematologic parameters.

The measurement of white blood cells is the most common method for assessing leucopenia, although lymphocytes value may also be used; to determine the cause of leucopenia other tests are required. The normal value or range for these indicators varies with both gender and age. Leucopenia is further categorized as mild, moderate or severe depending on how far a patient's white blood cells level resides below the normal range (UNAIDS 2003).

Normal values of white blood cell, the differential and red blood cell levels in group of human being:

Table 3: Normal values of white blood cells and their differential levels

Age (years)	White blood cells(x10^3/µL)	Neutrophils (x 10^3/µL)	Basophils (x 10^3/µL)	Eosinophils (x 10^3/µL)	Lymphocytes (x 10^3/µL)	Monocytes (x 10^3/µL)
10-15	5.5-14.5	1.50-7.50	0-0.20	0-0.50	1.50-5.00	0-0.80
15-20	4.5-13.5	1.80-7.00	0-0.20	0-0.50	1.20-5.00	0-0.80
Adult	4.3-10.0	1.80-7.00	0-0.20	0-0.50	1.00-4.80	0-0.80

(Source: http:// en.wikipedia.org/wiki/ hematological normal values, retrieved on 24th May 2013)

1.2.4. Monitoring of HIV disease progression

CD4 T cell are fundamental for the development of specific immune responses to infection, particularly intracellular pathogens. HIV largely infects activated cells causing the activated T-cells directly against the virus to be at greatest risk of infection (Stebbing J. *et al*, 2004). The CD4 T-cell count is the most significant predictor of disease progression and survival (Phillips AN et al, 2006) and the US department of health and human services(DHHS) ART treatment guidelines recommends treatment commencement be based on CD4 T-cell count in preference to any other single market. Use of CD4 count as means of monitoring efficacy is well established (Moore RD *et al*, 1999).

Immunological recovery is largely dependent on baseline CD4 count and thus the timing of ART initiation is important in order to maximize the CD4 T-cell response to therapy (Battegay M *et al*, 2006).

1.3. Clinical staging of HIV/AIDS for adults and adolescents

Primary HIV infection:

Asymptomatic and Acute retroviral syndrome

Clinical stage 1:

Asymptomatic and Persistent generalized lymphadenopathy (PGL)

Clinical stage 2:

Moderate unexplained weight loss, recurrent respiratory tract infections (sinusitis, bronchitis, otitis media, pharyngitis), recurrent oral ulcerations etc.

Clinical stage 3:

Conditions where a presemptive diagnosis can be made on the basis of clinical signor simple investigations such as HIV wasting syndrome, recurrent bacterial pneumonia, toxoplasmosis in the brain, extra pulmonary TB, esophageal candidiasis etc. conditions where confirmatory diagnostic testing is necessary: Meningitis, recurrent septicemia, any disseminated mycosis, cytomegalovirus (CMV) infection, lymphoma, cryptosporidiosis etc.

Table 4: CD4 levels in relation to the severity of immunosuppression

The severity of immunosuppression	**CD4 levels**
Not significant immunosuppresion	>500/mm^3
Mild immunosuppression	350-499/mm^3
Advanced immunosuppression	200-349/mm^3
Severe immunosuppression	<200/mm^3

(Source: http:// en.wikipedia.org/wiki/ CD4 levels and immunosuppression, retrieved on 24th May 2013)

1.4. Determination of CD4 in HIV patients

The determination of CD4 lymphocytes is currently a very important marker of HIV-induced immune impairment. It can assess the degree of immune deterioration and speed of progression towards AIDS, and can improve AIDS surveillance through CD4 cell count reporting. It can group HIV-seropositive naive patients into cohorts according to their baseline CD4 cell counts before initiating therapy. It can select the optimum timing for prophylaxis of opportunistic infections in AIDS. Finally, it can monitor the efficacy of antiretroviral and/or cytokine therapy or protective therapeutic vaccines. Therefore, the determination of CD4 cells is of crucial clinical importance for patients with HIV/AIDS (Mayo, 2009).

Flow cytometry represents the gold standard for accurate determination of CD4 cells. The high technical and financial expenditure involved in flow cytometric systems and protocols is the main reason for their currently low distribution in developing countries. However, the dramatic increase in HIV infections and AIDS in developing countries has led to new approaches aimed at improving this critical situation. Against this background, we evaluated a new flow cytometric concept for determination of CD4 cells by a highly simplified volumetric counting (SVC) method using a low-cost flow cytometer. A defined blood volume is measured directly after incubation with a single CD4-antibody with no further pre-analytic sample manipulation (Cassens *et al*, 2004).

1.5. Etiology of leucopenia in patients with HIV infection

The multiple cause of leucopenia in HIV infected patients can be categorized into those that arise from decreased leucocytes, ineffective leucocytes or increased white blood cell (WBC) destruction. Early in the HIV epidemic, Mycobacterium avium complex (MAC) disease was frequently observed in patients with AIDS and was a common cause of severe leucopenia in HIV infected patients (Richard *et al*, 2001)

Parvovirus B19 also induces leucopenia in HIV-infected patients, but there is evidence that the incidence is similar to that seen in HIV-negative individuals. Cytomegalovirus (CMV) infection is commonly seen in patients with AIDS and is associated with reduced leucocytosis.

Other pathogens, such as Pneumocystis carinii and Leishmania species, may cause leucopenia in patients with HIV infection in rare circumstances because of frequency of recurrent opportunistic infections in patients with AIDS, leucopenia of chronic disease is the most common type of leucopenia seen in this population. However, leucopenia of chronic disease is under diagnosed and often inappropriately treated and misunderstood. In this condition, RBCs are typically normochronic and normocytic, but during chronic or recurrent opportunistic infections they may become hypochromic and microcytic

(http://scholar.google.com/scholar?q=etiology+of+leucopenia&btnG=&hl=en&as_sdt=0%2C5, retrieved on 09th May 2013).

Leucopenia is an abnormal reduction of circulating white blood cells, especially the granulocytes. The term leucopenia is often used interchangeably with neutropenia. It may result from reduced production of white blood cells or increased utilization and destruction, or both. Infection, drugs, malignancy, megaloblastosis, hypersplenism and immunoneutropenia are responsible for most cases of neutropenia. Primary neutropenia is very rare. Sometimes, particularly in children, primary neutropenia is hereditary and may be associated with other developmental defects. The major danger of neutropenia is the risk of infection. Management requires identification of the cause and effective antimicrobial therapy, especially when serious systemic infection is present (Richard *et al*, 2001).

1.6. Effect of ARV on HIV positive patients

Antiretroviral drugs are medications for the treatment of infection by retroviruses, primarily HIV. When several such drugs, typically three of four, are taken in combination, the approach is known as highly active antiretroviral therapy, or HAART (Dybul M et al, 2002). 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 and preventing its further elongation. 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 final assembly of new virion. Integrate inhibitors inhibit the enzyme integrase, which is responsible for integration of viral DNA into the DNA of the infected cell. 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.

Maturation inhibitors inhibit the last step engage processing in which the viral capsid polyprotein is cleaved, thereby is blocking the conversion of the polyprotein into the mature capsid protein (p24). Because these viral particles have a defective core, the virions reseased consist mainly of non- infectious particles. There are no drugs in this class currently available, though two are under investigation, bevirimat. AV-HALTs (Antiretroviral Hyper Activation Limiting Therapeutics or virostatics) combine immune modulating and antiviral properties to inhibit a specific antiviral target while also limiting the hyper-elevated state of immune system activation drive to disease progression (Mayo, 2009).

1.6. Epidemiology of HIV/AIDS and leucopenia

In 2002, about 5 million people worldwide became infected with HIV, bringing the total number of persons living with the virus to approximately 42 million and about 86% live with leucopenia. Projections for the next 10 years suggest that the situation will become even more serious, with possibly 100 million infected individuals. About 70% of the HIV-infected persons worldwide reside in sub-Saharan Africa and up to 70% of all hospital beds in African countries are currently occupied by AIDS patients. In 2002 alone, about 3.5 million persons were newly infected in sub-Saharan Africa. In contrast, only 10 000 of about 4 million HIV-infected individuals in South Africa (0.25%) and only 30 individuals out of 1 million infected persons in Malawi (0.003%) can afford access to HIV-related diagnostics and treatment at current prices (http://scholar.google.com/scholar?hl=en&as_sdt=0,5&q=epidemiology+of+leukopenia.pdf, retrieved on 05 June 2013).

This situation not only represents a serious human and ethical catastrophe, but also dramatically diminishes the economic situation in countries with high HIV prevalences. Studies have demonstrated that programmes for the affordable diagnosis, treatment and prophylaxis of

HIV/AIDS in Africa are highly efficient in improving the dramatic situation. Recently, some pharmaceutical manufacturers have distinctly reduced the prices of their HIV/AIDS drugs in developing countries. Therefore, the need for affordable diagnostic tools for the treatment of AIDS in developing countries has also increased (Cassens *et al,* 2004).

1.7. Treatment and prevention leucopenia in HIV patients

Treatments to correct adenosine deaminase deficiency presently are being actively investigated. Lymphocyte counts below 1109 /L accompany many acute infections, particularly those characterized by granulocytosis. Lymphopenia is characteristic of HIV- infected individuals, and an absolute reduction in CD4- positive T lymphocytes is one of the earliest immunological consequences of HIV infection. Agents that mimic the genetic defect in adenosine deaminase deficiency were developed for the treatment of low-grade lymphoproliferative disorders. They act by irreversibly binding to adenosine deaminase (deoxycoformycin) or by resisting deamination in the purine salvage pathway (2-chloroadenosine). These agents can lead to severe lymphopenia lasting 1–2 years
(http://scholar.google.com/scholar?q=treatment+and+prevention+leucopenia+in+HIV+patients.pdf&btnG=&hl=en&as_sdt=0%2C5, retrieved on 04th May 2013).

Fludarabine, which inhibits ribonucleotide reductase, is now a commonly used agent for the treatment of chronic lymphocytic leukemia. Its use can lead to severe T-cell immunodeficiency and lymphopenia resulting in occasional infections with Pneumocystis carinii or cytomegalovirus.

The first task for Damian Marron at Cytheris will be to guide the acceleration of the development of its lead compound CYT107 (glycosylated r-hIL-7) with pivotal studies in two targeted indications with urgent medical needs and no current treatments:

- Progressive Multifocal Leukoencephalopathy (PML) in severely leucopenic patients; an orphan indication with a one year survival of around 50 per cent that affects approximately 4,000 people year in the US and EU alone
- Reduction of severe complications in Immune Non-Responders with HIV (HIV-INR) controlled by anti-retroviral treatment; 15-25 per cent of HIV patients are leucopenic despite optimal treatment (HAART) leading to a significant increase in risk of death and serious complications.

CYT107 also holds great promise in other leucopenic conditions such as idiopathic leucopenia or cancer associated leucopenia. “I am delighted to be taking on responsibility for leading Cytheris in the next stage of its development,” said Damian Marron. “Cytheris has already generated a significant body of clinical and pre-clinical evidence demonstrating the ability of CYT107 to reverse leucopenia and restore immune function. I look forward to allying my experience with

that of the Cytheris team, its board and investors to build further value and bring treatments to patients with serious and under-treated conditions."

Olivier Martinez, chairman of the Cytheris board added: "We congratulate Damian Marron on his appointment. He brings over 25 years of biotechnology and industry experience to the role, especially from his last four years as CEO with Trophos. There Damian was instrumental in establishing and executing on a strategy that included an innovative option acquisition agreement with Actelion, undertaking a pivotal registration program in an orphan indication and ensuring Trophos was well-financed to advance its programs."(UNAIDS, 2008).

❖ **About Cytheris**

C**y**theris is a privately held clinical-stage biopharmaceutical company focused on treating lymphopenia driven diseases. The company's lead drug candidate, CYT107 (glycosylated r-hIL-7), is in clinical development for two targeted indications with urgent medical needs and no current treatments:

- Progressive Multifocal Leukoencephalopathy (PML) in severely lymphopenic patients; an orphan indication with a one year survival of around 50 per cent that affects approximately 4,000 people year in the US and EU.
- Reduction of complications in Immune Non-Responders with HIV controlled by anti-retroviral treatment (HIV-INR); 15-25 per cent of HIV patients are lymphopenic despite optimal treatment (HAART) leading to a significant increase in risk of death and serious complications.

CYT107 is a critical growth factor for immune T cell recovery and enhancement. Clinical trials conducted on more than 240 patients in Europe, North America, South Africa and Taiwan have demonstrated the ability of CYT107 to expand and protect CD4+ and CD8+ T-cells in various pathologic conditions as well as a consistent safety and tolerability profile (http://www.cytheris.com/pubs/pdf/Chapter4, retrieved on 14th May 2013).

PART II: EXPERIMENTAL STUDY

CHAP II. MATERIALS AND METHODS

2.1. Materials and reagents

- **Materials**

- Hematology mixer
- QBC Sysmex500i
- Distilled water
- Computer with printer
- Work station area and QBC Accu Tubes
- BD FACS Count, The BD FACS count system, for use with BD FACS Count CD4 reagents, is an automated instrument and reagent system designed specifically for enumerating the absolute cell counts of CD4 T lymphocytes and the percentage of lymphocytes that are CD4 T lymphocytes in unlysed whole blood (CD4 counts and CD4 percentages). The reagents are intended for in vitro diagnostic use on a BD FACS Count instrument.
- Coring station: opens the sealed reagent and control tubes to prepare them for use.
- Top-MIX94323: is a vibrating machine that mix the sample with the reagents
- Electronic Pipettes: accurately delivers 50µL of fluid. This pipette is automated and programmed.
- Work station: holds blood specimens and operating supplies during sample and control preparation.

- **Reagents**

✓ Four types of reagents are used with Sysmex500i in Hematology analyzer. All of them are specialized reagents for use in Sysmex500i equipment. Please follow the warnings for handling and using each of the reagents correctly.

- Cell pack reagent: diluents for use in hematology analysers. The performance of Sysmex500i equipment cannot be guaranteed if anything else is used for dilution.
- Stromatolyser reagent: used to stain the leukocytes in diluted and lysed blood sample. It serves for the determination of 5part differential count (Neut, Lymph, Mono, Eo, Baso) and WBC count with selected sysmex500i hematology analyzers.
- Sulfolyser reagent: is a cyanide-free reagent used for the determination of hemoglobin.
- Cell clean reagent: is a strong alkaline detergent to remove lysing reagents, cellular residuals and blood proteins remaining in the hydraulics of Sysmex Automated Hematology analysers.
- e–CHECK controls reagent (high, medium, low): is a quality control material. Quality control is performed in order to monitor an instrument's performance over time (Kobe, 2011).

✓ The next reagents are used in flow cytometry method

- Fax clean reagent
- Fax lens reagent
- Fax flow reagent
- CD4 reagents (fixative solution and CD4 control)

2.2. Methods

2.2.1. Hematology Analyzer

2.2.1.1. Principles

Method of counting and volumetric sizing based on the detection and measurement of changes in electrical resistance produced by a particle suspended in a conductive liquid. A blood sample is diluted in saline, which is a good conductor of electrical current. The blood samples are injected in Sysmex500i which is automatically scanned.

2.2.1.2. Procedures

- Venous blood collected in vacutainer tube with EDTA are used and stored at room temperature
- To put a tube on the mixer
- To remove a tube from the mixer and make the sample aspired by the Sysmex500i machine.
- To print the results. Analyzer door was opened and Accu Tube was removed.

2.2.2. Flow Cytometry

2.2.2.1. Principles

Running of the samples on the BD FACS Count instrument, a build-in screen displays instructions for operation. The following procedure requires minimal sample handling.

2.2.2.2. Procedures

- Add the reagents in the whole blood
- Add a fixative solution to the reagent tubes
- Run the sample on the instrument
- Results include CD4 counts and CD4 percentages are printed immediately after samples are run.

CHAPTER III: RESULTS PRESENTATION, INTERPRETATION AND DISCUSSION

3.1. Results presentation and interpretation

In this chapter the results of the study are described and analyzed according to the data. The results describe the information on the white blood cells with their differential and red blood cells levels in HIV positive patients:

3.1.1. WBC level in two different patients under ARVs and without ARVs

The table 5 shows the white blood cells level comparison among patient under treatment and those not yet starting treatment.

Table 5: Comparison of WBC level in the patients under ARV and those without ARV

WBC ranges ($x10^3/\mu L$)	**ARV**		**NO ARV**		**TOTAL**	
	Frequency(n=68)	%	Frequency(n=68)	%	Frequency(n=136)	%
> 10.0	0	0	0	0	0	0
10.0 – 8.0	3	2.2	6	4.4	9	6.6
8.0 - 6.0	15	11.0	24	17.6	39	28.6
6.0- 4.3	23	17.0	25	18.4	48	35.4
< 4.3	27	19.8	13	9.6	40	29.4
Total	**68**	**50**	**68**	**50**	**136**	**100**

A total of 136 HIV positive patients were enrolled in this study; 50% received ARV and the same percentage did not start this treatment. Normal WBC value is in the range between 4.3 $x10^3/\mu L$ and 10.0 $x10^3/\mu L$ but we found that a great percentage (19.8%) of patients under ARV have lower level of WBC which explain leucopenia than patients not yet starting ARV. Leucopenia is more observed in patients under ARVs because of its consumption.

3.1.2. Neutrophils level in two different patients under ARVs and without ARVs

The table 6 will show the level of neutrophils in HIV patients under ARV and other without ARV.

Table 6: Comparison of neutrophils level in the patients under ARV and those without ARV

Neutrophils ($x10^3/\mu L$)	**ARV**		**NO ARV**		**TOTAL**	
	Frequency(n=68)	%	Frequency(n=68)	%	Frequency(n=136)	%
> 7.00	0	0	0	0	0	0
7.00 - 5.00	0	0	0	0	0	0
5.00 – 3.00	14	10.3	16	11.8	30	22.1
3.00 – 1.00	37	27.2	47	34.5	84	61.7
< 1.00	17	12.5	5	3.7	22	16.2
Total	**68**	**50**	**68**	**50**	**136**	**100**

The table 6 show that in 136 HIV positive patients, in the patients who receive ARV 12.5% have neutropenia (lower level of neutrophils) but in other who did not receive ARV 3.7% have neutropenia means that this kind of disease is more present in patient under ARV while the normal range is between 7.00 $\times 10^3$ µL and 1.00 $\times 10^3$ µL. This decreasing is caused by ARVs consumption.

3.1.3. Lymphocytes level in two different patients under ARVs and without ARVs

The table 7 show the comparison of lymphocytes level in HIV positive patients under ARV and those not yet starting ARV.

Table 7: Comparison of lymphocytes level in the patients under ARV and those without ARV

Lymphocytes ($x10^3/\mu L$)	**ARV**		**NO ARV**		**TOTAL**	
	Frequency(n=68)	%	Frequency(n=68)	%	Frequency(n=136)	%
> 4.80	0	0	0	0	0	0
4.80 – 3.80	0	0	0	0	0	0
3.80 – 2.80	11	8.1	16	11.8	27	19.9
2.80 – 1.00	51	37.5	52	38.2	103	75.7
< 1.00	6	4.4	0	0	6	4.4
Total	**68**	**50**	**68**	**50**	**136**	**100**

Among 136 HIV positive patients 50% of them have started ARV and others did not start those drugs. It was found that in patients under ARV 4.4% suffer lymphopenia (Lower level of

lymphocytes than the normal which is between 4.80 $\times 10^3 \mu L$ and 1.00 $\times 10^3 \mu L$) and any patient without treatment present this kind of disease. Lymphopenia is caused by ARVs consumption.

3.1.4. Monocytes level in two different patients under ARVs and without ARVs

The table 8 shows how monocytes are compared in HIV patients under ARV and without ARV

Table 8: Comparison of monocytes level in the patients under ARV and those without ARV

Monocytes ($x10^3/\mu L$)	**ARV**		**NO ARV**		**TOTAL**	
	Frequency(n=68)	%	Frequency(n=68)	%	Frequency(n=136)	%
> 0.80	3	2.2	5	3.7	8	5.9
0.80 – 0.50	20	14.7	21	15.4	41	30.1
0.50 – 0.20	39	28.7	42	30.9	81	59.6
0.20 – 0.01	6	4.4	0	0	6	4.4
< 0.01	0	0	0	0	0	0
Total	**68**	**50**	**68**	**50**	**136**	**100**

In the total number of HIV positive patients, 68 patients have started ARV and the same patients did not start this treatment. Normal range of monocytes in blood is between 0.01 $x10^3/\mu L$ and 0.80 $x10^3/\mu L$, in both patients there is no case of lower level in monocytes but high level is more present in HIV patients without ARV which have 7.4% than 4.4% present in patients that have starting ARV. This increasing could be influenced by other unknown infections.

In HIV patients under ARV and without ARV we found no case of monocytopenia (lower level of the normal).

3.1.5. Eosinophils level in two different patients under ARVs and without ARVs

The table 9 show the comparison of eosinophils in patients under ARV and those not yet starting ARV.

Table 9: The distribution of eosinophils level in the patients under ARV and those without ARV

Eosinophils ($x10^3/\mu L$)	**ARV**		**NO ARV**		**TOTAL**	
	Frequency(n=68)	%	Frequency(n=68)	%	Frequency(n=136)	%
> 0.50	14	10.3	31	22.8	45	33.1
0.50 – 0.30	11	8.1	16	11.7	27	19.8
0.30 – 0.10	23	16.9	11	8.1	34	25
0.10 – 0.01	17	12.5	10	7.4	27	19.9
< 0.01	3	2.2	0	0	3	2.2
Total	**68**	**50**	**68**	**50**	**136**	**100**

About 136 HIV positive patients, 50% of them are under ARV and others have not starting ARV. Normal value is between 0.01 $x10^3/\mu L$ and 0.50 $x10^3/\mu L$ but in HIV positive patients under ARV, 2.2% have lower level of eosinophils but any patients without ARV who has lower level of eosinophils.

3.1.6. Basophils level in two different patients under ARVs and without ARVs

The table 10 presents the comparison of basophils among patients under ARV and without ARV

Table 10: Comparison of basophils level in patients under ARV and those not yet stating ARV

Basophils ($x10^3/\mu L$)	**ARV**		**NO ARV**		**TOTAL**	
	Frequency(n=68)	%	Frequency(n=68)	%	Frequency(n=136)	%
> 0.20	0	0	0	0	0	0
0.20 – 0.15	0	0	0	0	0	0
0.15 – 0.05	3	2.2	0	0	3	2.2
0.05 – 0.01	37	27.2	31	22.8	68	50
< 0.01	28	20.6	37	27.2	65	47.8
Total	**68**	**50**	**68**	**50**	**136**	**100**

A group of 136 HIV positive patients, 50% were under ARV and others did not stated ARV. Normal range of basophils is between 0.01 $x10^3/\mu L$ and 0.20 $x10^3/\mu L$ but we found that 20.6%

patients under ARV have lower level of basophils and 27.2% patients without ARV are under normal level. This observation shows that there is no effect of ARV on basophils but the patients who did not starting ARV they tend to have lower level of basophils.

3.1.7. CD4 level in two different patients under ARVs and without ARVs

The table 11 and figure 8 show the immunosuppression among both HIV psitive patients under ARV and those without ARV

Table 11: Determination of immunosuppression level based on CD4 cell counts in HIV patients under ARV and those without ARV

CD4 cell /µL)	ARV		NO ARV		TOTAL	
	Frequency(n=68)	%	Frequency(n=68)	%	Frequency(n=136)	%
>500	38	27.9	52	38.2	90	66.1
350-499	7	5.2	16	11.8	23	17.0
200-349	18	13.2	0	0	18	13.2
<200	5	3.7	0	0	5	3.7
Total	**68**	**50**	**68**	**50**	**136**	**100**

The table 11 represents the number of CD4 levels in relation to the severity of immunosuppression: Among patients under ARV: >500 cells/ µL which explain the not significant immunosuppression were 27.9%, 350-499 $x10^3$ cells/ µL which explain the mild immunosuppression were 5.2% and advanced immunosuppression had CD4 range between 200 cells/ µL and 349 cells/ µL were 13.2% then severe immunosuppression were <200 cells/ µL with 3.7% but in HIV patients without ARVs, we found the not significant immunosuppression and mild immunosuppression which were respectively: >500 cells/ µL were 38.2% and 350-499 cells/ µL which were 11.8% but no case of advanced and severe immunosuppression. We found that the immunosuppression was higher in HIV patients under ARVs than HIV patients without ARVs caused by the high CD4 levels presented in patients without ARVs than in patients under ARVs.

3.2. Results discussion

The main goal of this study was to compare the blood cell level specifically white blood cell with their differential and CD4 level in HIV positive patients under ARVs and those not yet starting ARVs.

In this study, the patients with HIV positive aged 10 years and over, who were in monitoring or requested for CD4 cell counts were diagnosed for white blood cell level, their level of differential and CD4 level where the decreasing of white blood cell from the normal level between (10-4.3 $\times 10^3 \mu L$) discovered by UNAIDS to the lower level that define leucopenia, was found more in patients under ARVs at 19.80% than in patients without ARVs which was at 9.6% compared to the normal range.

However mild with the range between 4.0 – 3.5 $\times 10^3 \mu L$ discovered by UNAIDS and moderate with the range between (3.5 – 3.0 $\times 10^3 \mu L$) done by UNAIDS were 9.5% to 8.7% respectively and in all study population, 29.4% were leucopenic to 70.6% who had normal white blood cell levels (4.3-10.0 µL) that was confirmed by the study of an American Odani *et all* in 2005 and in patients under ARV, leucopenia occurred at 19.8% while for the patients who did not receive ARV was at 9.6%, this showed that the patients under ARV were more leucopenic (19.8%) than those without ARV (9.6%) and severe leucopenia was more predominant in patients under ARV 10.7% while in patients without ARV 0.9%, this may be due to severe immunosuppression which was marked to side effect of ARV, all of those normal range compared to our results have been studied by Mayo in 2009.

These results showed that ARVs have the effect on hematological parameters such as WBC and the differentials (neutrophils, monocytes, lymphocytes, eosinophils and basophils) compared to the study of an England Dybul M *et al* in 2002, whereas based on WBC level to the patients under ARV leucopenia was at 19.8% while for patients without ARV was at 9.6%. For lymphocytes level in HIV patients under ARV, lymphopenia was at 4.4% but in patients not yet starting ARV was at 0%. According to neutropenia studied in 2001 by Balt CA and Nixon H in HIV positive patients under ARVs was at 12.5% while in patients without ARV was at 3.7%. From the results of eosinophils showed that the decreasing in lower level was at 2.2% in HIV patients under ARV and 0% in HIV positive patients not yet starting ART, we have also found that for monocytes there was any effect of ARV because in patients under ARV monocytopenia tend to increase from 4.4%% to 0% in patients without ARV but it's had been found that for basophils in HIV patients under ARVs and without ARVs was a special case, whereas basophils level was lower in patients under ARVs with 20.6% of the total patients than in patients without ARVs with 27.2% of the total patients, that explained that ARVs has no any side effect on basophils level but ARVs have an effect of increasing the level of basophils from the normal

range (0.20 – 0.01 10^3µL) showed by UNAIDS to the highest. According to CD4 level and immunosuppression which was defined based on CD4 level studied by an American Mayo in 2009, whereas the not significant, the mild, the advanced and the severe were respectively at the ranges of >500 cells/mm^3, 350 – 499 cells/mm^3, 200 – 349 cells/mm^3 and <200 cells/mm^3. we found that the immunosuppression was more found in patients under ARVs than those without ARVs >500 cells/ µL which explain the not significant immunosuppression were 27.9%, 350-499 $x10^3$ cells/ µL which explain the mild immunosuppression were 5.2% and advanced immunosuppression had CD4 range between 200 cells/ µL and 349 cells/ µL were 13.2% then severe immunosuppression were <200 cells/ µL with 3.7% but in HIV positive patients without ARV: >500 cells/ µL were 38.2%; 350-499 cells/ µL were 11.8 but no case of advanced and severe immunosuppression, means that the immunosuppression is higher in HIV positive patients under ARVs than in HIV patients without ARVs.

CHAPTER IV: CONCLUSION AND RECOMMENDATIONS

4.1.Conclusion

Hematologic parameters, white blood cells count (WBC) with their differential cell count, among others are important monitoring tools for assessing treatment and prognosis in HIV/AIDS. Apart from the CD4 count, a full blood count is the commonest pre-treatment investigation done for people living with HIV.

The use of antiretroviral drugs could positively or negatively affect these parameters, depending on the choice of combination used. Although many drugs used for the treatment of HIV-related disorders are myelo-suppressive, severe cytopenia is most often related to the use of zidovudine. Hence the need to review these parameters in a group of treatment-naïve HIV-infected patients only cannot be overemphasized.

Some hypothesis was been presented: White blood cells level could decrease more in HIV positive patients under ARVs than those without ARVs, the differential could vary in both HIV patients started ARVs and others not yet starting ARVs then HIV positive patients under ARVs could have lower CD4 level than those without ARVs.

The aim of this study was to compare white blood cells with their differential levels in HIV positive patients under treatment and those not yet starting treatment and to determine the immunosuppressant level based on CD4 cell count in HIV patients under treatment and those without treatment.

From this analysis there was a great decrease of WBC in HIV patients under ARVs than those without ARV where in 136 patients, 19.8% of the total patients under ARVs were leucopenic but 9.6% of the total patients without ARVs were leucopenic, this show that ARVs have side effect on HIV disease and cause leucopenia, neutrophils level decrease also in HIV patients under ARVs where was presented in lower level than a normal 12.5% of the total more than in those without ARVs were 3.7%.

Lymphocytes normal level decrease more in patients under ARVs with 4.4% of the total patients than in patients without ARVs that were 0%, monocytes normal level had more decreasing in HIV patients under ARVs by 4.4% of the total patients than in patients without ARVs by 0%, eosinophils normal level decrease also in patients under ARVs with 2.2% of the total more than in patients without ARVs with 0% but there was a particular case on basophils normal level where in patients under ARVs 20.6% of the total patients were less than in the patients not yet stating ARVs that were 27.2% of the total patients as the HIV disease progressed.

The immunosuppression was higher in patients under ARVs than without ARVs where Among patients under ARV: >500 cells/ µL which explain the not significant immunosuppression were 27.9%, 350-499 $x10^3$ cells/ µL which explain the mild immunosuppression were 5.2% and

advanced immunosuppression had CD4 range between 200 cells/ µL and 349 cells/ µL were 13.2% then severe immunosuppression were <200 cells/ µL with 3.7% but in HIV positive patients without ARV: >500 cells/ µL were 38.2%; 350-499 cells/ µL were 11.8 but no case of advanced and severe immunosuppression, means that the immunosuppression is higher in HIV positive patients under antiretroviral treatment than HIV positive patients without antiretroviral treatment. All of the normal range that we talked about have been done and presented by UNAIDS.

In our study, 29.4% were leucopenic to 70.6% who had normal white blood cell levels and in patients under ARV leucopenia occurred at 19.8% while for the patients who did not receive ARV were at 9.6%, this showed that the patients under ARV were more leucopenic and shows that white blood cell level in patients under ARV is lower than those without ARV.

4.2.Recommendations

Like any scientific work must be completed to make the results more relevant, some recommendations are formulated:

- **To researchers**

To discover ARVs in which daesn't any impact on other Blood cell level

- **To medical staff**

To screen CD4 counts and other blood cell level for the test of blood cell level

- **To person living with HIV/AIDS**

To consult regularly the physicians for check up of disease progression and perform all measures which may help the increasing of their immunity.

- **To INES – Ruhengeri**

To have memorandum of understanding with different Hospitals in order to facilitate the students in their medical research.

REFERENCES

A) Books:

1. Balt CA, Nixon H (2001). *"Complication of HIV: lipodystrophy, leucopenia and bone diseases"*, Nurse Practice forum. Dec; 10(2): 187-205.

2. Battegay M, Nuesch R, Hirschel B, Kaufman GR (2006). *"Immunological recovery and antiretroviral therapy in HIV-1 infection. Lancet infectious diseases"*, 4(6): 274-282.

3. Bernard A (2008). "*BD FACS Count System User's Guide*", Oxford University Press; 1989:796-797.

4. Clark (2009): *"A practical approach to the differential diagnosis and evaluation of the adult patient with leucopenia"*. Medicine clinical of North American; 5(7): 124-132.

5. Doukas MA (2007*)* "*Centrifuge hematology system. Scientific and medical supplies Ltd*. Colombia University", 7(5): 176-189).

6. Kobe (2011) "*Automated hematology analyzer*". Wakinohama-Kaigandori, Chuo-ku. P 4-1 to 4-7.

7. Mayo (2009). Leucopenia in adult: a contemporary approach to diagnosis. Mayo clinical procedures; 69:895-902.

8. Mildivan D. (2003). *"Biomedical science explained: Hematology."* 7th edition. P 42-46.

9. Phillips AN, Lundgren JD (2006)." *The CD4 lymphocyte count and risk of clinical progession*. Current opinion in HIV & AIDS, HIV Medicine" 1(4):51-58.

B) Journals and articles:

1. Akinbami ,A., Oshinaike ,O., Adeyemo ,T., Adediran , A., Dosunmu , W., Dada , M., Durojaiye ,I., Adebola, A., and Oshunkalu, V.,2010., *Hematologic Abnormalities in Treatment-naïve HIV patients*.,(Department of hematology and Blood Transfusion, Lagos State University college of Medicine, Lagos, Nigeria), P3-4.

2. Amitic Ramezani, Arezoo Aghakhani (2008). *Leucopenia prevalence and related factors in HIV patients*. (A Cohort study. Iranian journal of pathology), P1-3.

3. Cassens ,U., Göhde ,W., Kuling,G., Gröning,A., Schlenke,P., Henin,Y,Greve,B.,2004., *Simplified volumetric flow cytometry allows feasible and accurate determination of CD4 T lymphocytes in immunodeficient patients worldwide.*(Institute of Transfusion Medicine, Germany), P1-2.

4. Dybul M, Judith S. Currier, MD, and Diane V. Havlir, MD., 2002. *Complications of HIV Disease and Antiretroviral Therapy*. (University of California San Francisco), P4.

5. Fiebige, N., 2004. *Lymphopenia and autoimmune diseases*. (Center for Molecular Medicine, Clinical Research Group III; and Department of Internal Medicine III and Institute for Clinical Immunology, University of Erlangen-Nuremberg, Germany), P3-5.

6. Florence E, Dreezen C, Schrooten W, Van Esbrek M, Kestens L, Fransen K, Dee Roo A, Colebunders R (2004). *The role of non-viral load surrogate markers in HIV-positive patient monitoring during antiviral treatment*. (International Journal of Std&Aids), P1.

7. Hoffman R, Olsen BE, Lie RT (2000). *Leucopenia in HIV patients: association with micronutrients status and infection.* (European journal of clinical nutrition), P2-3.

8. Mocroft A. *et al*, 1999) in references page we must have: Mocroft A, Henin,Y., Reichelt, , Kestens L, Fransen K (1999). *Leucopenia identification* (American journal of medicine), P1-3.

9. Moore RD, Bellesso M, Costa SV, Fischer Chamone D.A, Dorlhiac Llacer P.E (1999). *Screening for the outpatient treatment of febrile neutropenia*. (Brazilian journal of haematology), P4-5.

10. Odani S, Belperio PS, Rhew DC (2005). *Prevalence and outcomes of leucopenia in individuals with human immunodeficiency virus: a systematic review of the literature*. (American journal of medicine), P2-4.

11. Richard , Barton SE, Dietrich M, Proenca R, Colebunders R, Pradier C,D'Arminio Monforte A, Ledergerber B, Lundgren JD. Group ESIDA (2001). *Leucopenia is an independent predictive marker for clinical prognosis in HIV infected patients from across Europe.* (Journal of acquired immune deficiency Syndromes), P5.

12. RS Ferri, Bellesso M, Costa S.F, Fischer Chamone D.A, Dorlhiac Llacer P.E (2010). *Screening for the outpatient treatment of febrile neutropenia*. (Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo – USP, São Paulo (SP), Brazil), P2-3.

13. Stebbing J, Gazzard B, Douek DC (2004). *Mechanisms of disease – Where does HIV live?*, (New England journal of Medicine), P2.

14. UNAIDS (2003*). Global AIDS E report on the epidemic*. (3rd global report. Geneva 26-switzerland), P1.

15. UNAIDS (2008), *Report on the global AIDS Epidermic2008*. [UNAIDS 11th anniversary, special edition, Geneva (Switzerland), UNAIDS], P1-2.

C) Electronic sources:

1. http://scholar.google.com/scholar?q=treatment+and+prevention+leucopenia+in+HIV+patients.pdf &btnG=&hl=en&as_sdt=0%2C5, retrieved on 04th May 2013.

2. http://scholar.google.com/scholar?q=etiology+of+leucopenia&btnG=&hl=en&as_sdt=0%2C5, retrieved on 09th May 2013.

3. http://scholar.google.com/scholar?hl=en&as_sdt=0,5&q=epidemiology+of+leukopenia.pdf, retrieved on 05 June 2013.

4. http://scholar.google.com/scholar?q=Neutropenia+in+HIV+positive+patients%28pdf%29&btnG= &hl=en&as_sdt=0%2C5, retrieved on 05 June 2013.

5. http//en.Wikipedia.org/Structure of blood cells, retrieved on 16th June 2013.

6. http//en.Wikipedia.org/components of blood cells, retrieved on 16th June 2013.

7. Patrick Sullivan (2007). Leucopenia and survival in patients with HIV infection and new guidelines for the management of leucopenia. *http//www.jaids.com*,retrieved on 05th April 2013).

8. http//www.ncbi.nml.nih.gov/pubhem/12308654, retrieved on 29th June 2013.

9. http://www.cytheris.com/pubs/pdf/Chapter4, retrieved on 14th May 2013.

10. http//en.Wikipedia.org/Components of Blood cell.pdf, retrieved on 24th June 2013.

APPENDICES

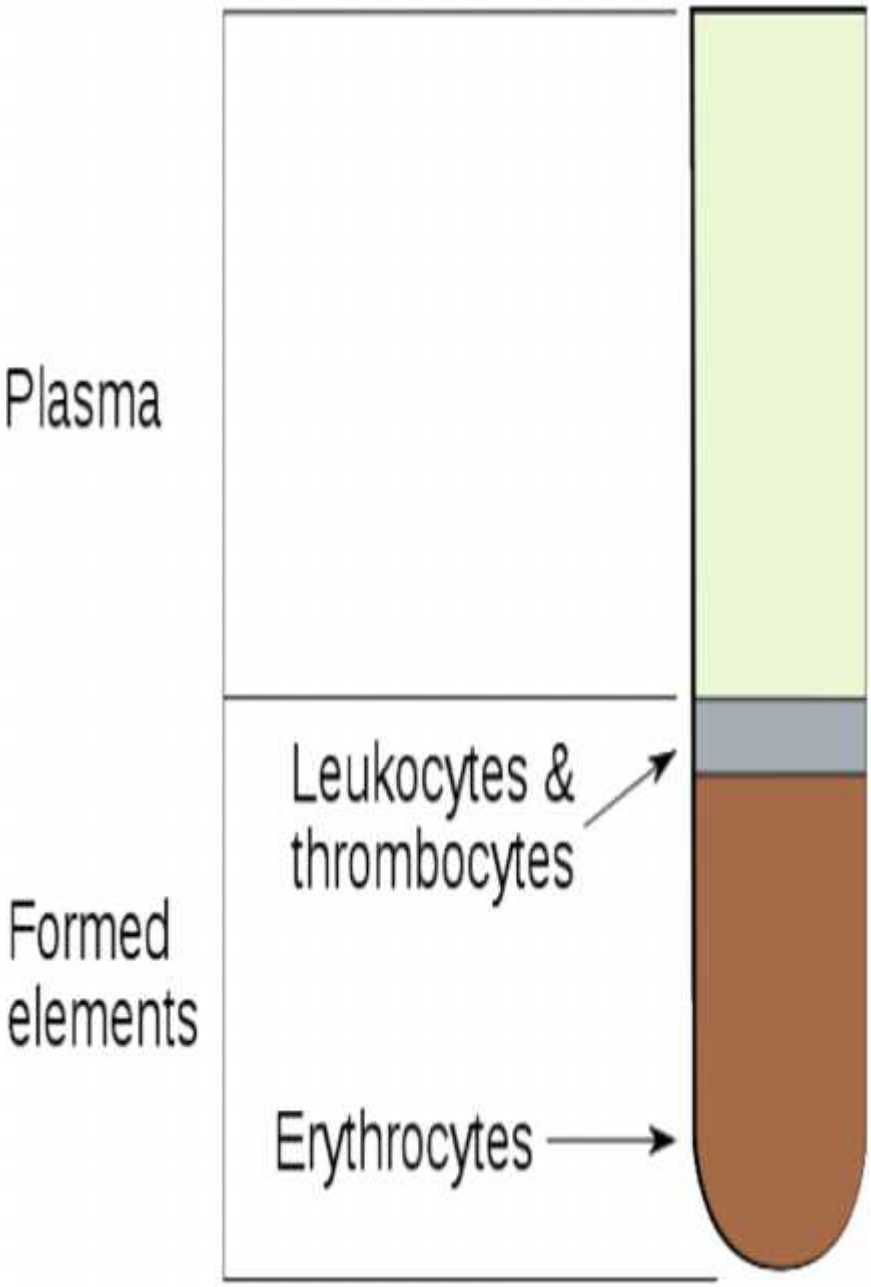
Appendix 1: WBC and their differential analysis in hematology room at GDHL

(Source: Photo taken by the author at GDHL in hematology room, on 08th June 2013)

Appendix 2: CD4 count analysis in CD4 room at GDHL

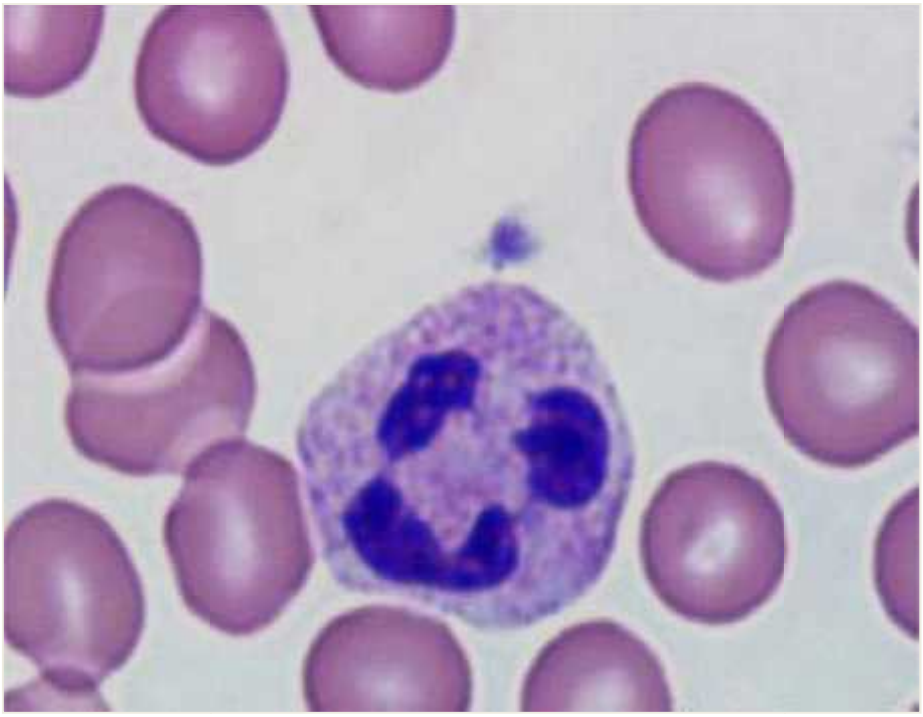
(Source: Photo taken by the author at GDHL in CD4 room, on 10th June 2013)

Appendix 3: Components of Blood cell



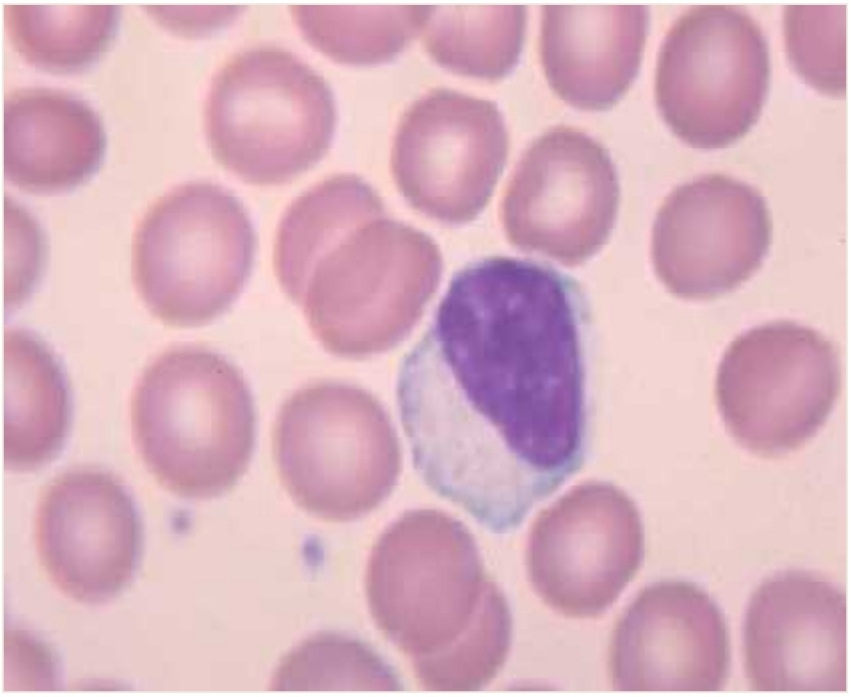
(Source: http//en.Wikipedia.org/Components of Blood cell.pdf, retrieved on 24th June 2013)

Appendix 4: Morphology of neutrophils



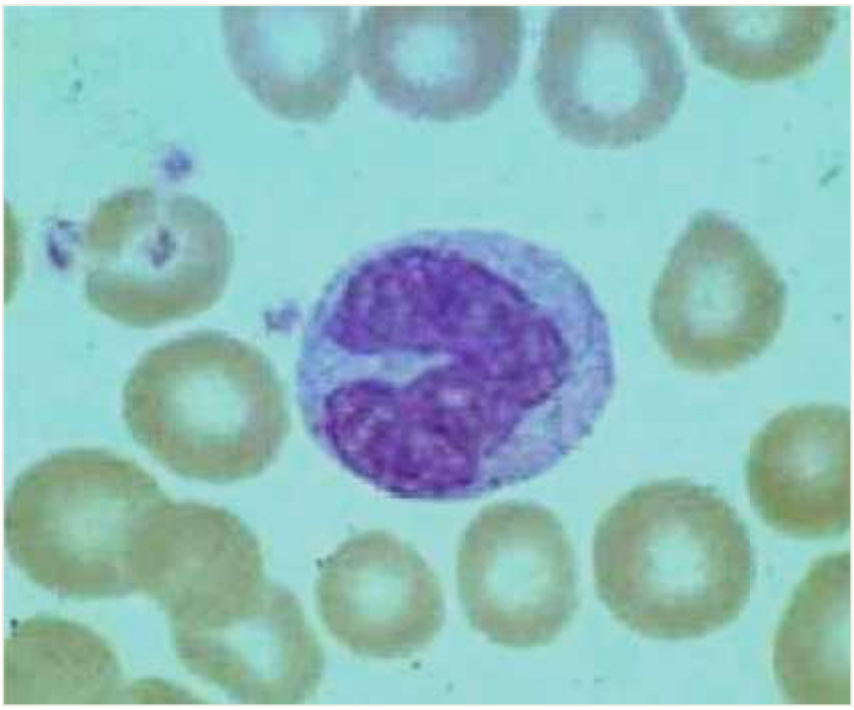
(Source: http//en.Wikipedia.org/Morphology of blood cells, retrieved on 16th June 2013)

Appendix 5: Morphology of lymphocytes



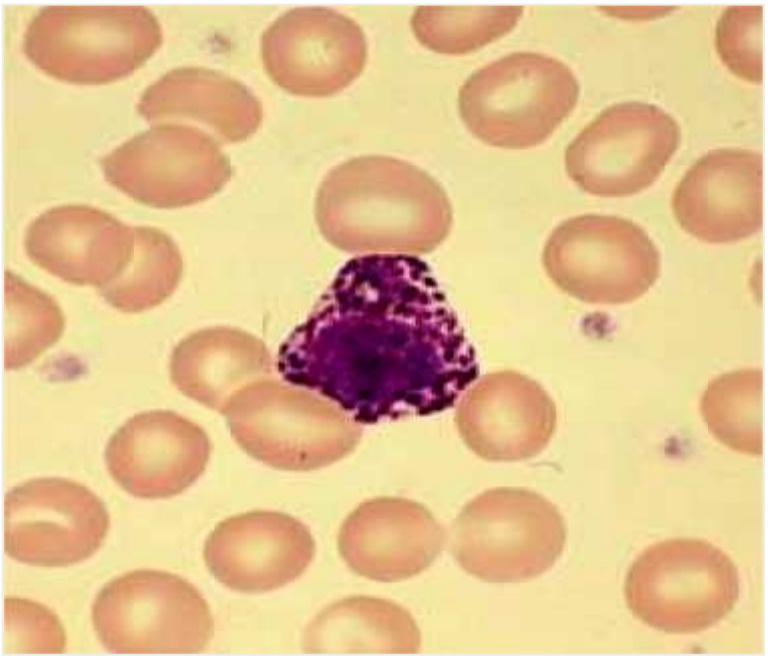
(Source: http//en.Wikipedia.org/Morphology of blood cells, retrieved on 16th June 2013)

Appendix 6: Morphology of monocytes



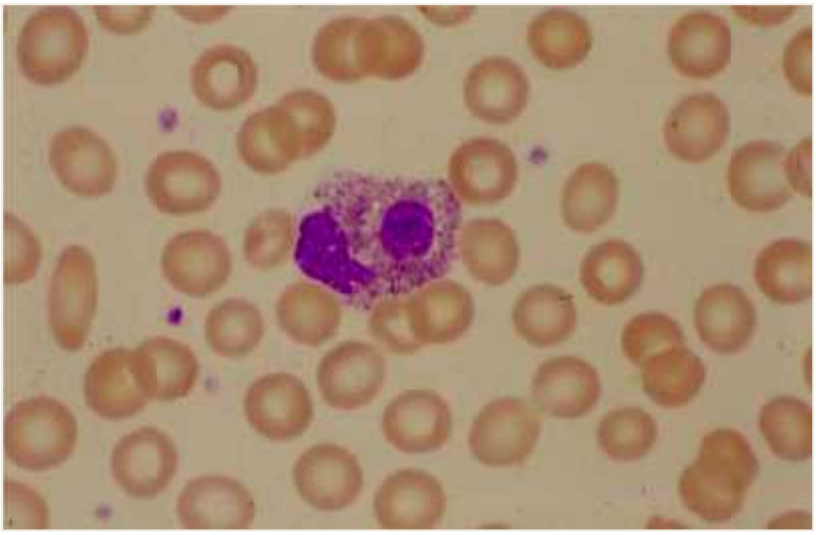
(Source: http//en.Wikipedia.org/Morphology of blood cells, retrieved on 16th June 2013)

Appendix 7: Morphology of basophils



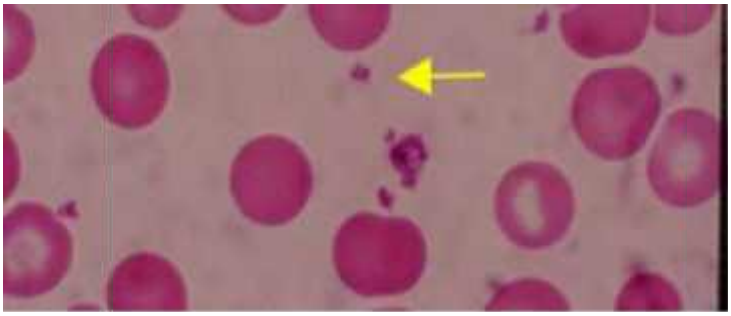
(Source: http//en.Wikipedia.org/Morphology of blood cells, retrieved on 16th June 2013)

Appendix 8: Morphology of eosinophils



(Source: http//en.Wikipedia.org/Morphology of blood cells, retrieved on 16th June 2013)

Appendix 9: Morphology of Platelets



(Source: http//en.Wikipedia.org/Morphology of blood cells, retrieved on 16th June 2013)