# CHANGES IN SERUM LEVELS OF HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS, ERYTHROPOIETIN AND PRO-INFLAMMATORY CYTOKINES IN HIV-INFECTED PATIENTS AT KENYATTA NATIONAL HOSPITAL-KENYA

JACKSON IRERI MRAMA (MSc.) I84/25779/2011

# A THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN MEDICAL BIOCHEMISTRY IN THE SCHOOL OF PURE AND APPLIED SCIENCES OF KENYATTA UNIVERSITY

**JANUARY, 2021** 

# DECLARATION

<sup>•</sup>I, Jackson Ireri Mrama, duly declare that this thesis is my original work and has not been presented for a degree in any other university or for any other award'

Mr. Jackson Ireri Mrama I84/25779/2011 Department o Biochemistry, Microbiology and Biotechnology Signature......Date..... Supervisors

We confirm that the work reported in this thesis was carried out by the candidate under our supervision

#### **Prof. Joseph J.N, Ngeranwa**

Department of Biochemistry, Microbiology and Biotechnology Kenyatta University Signature...... Date......

#### Dr. David N, Mburu

Department of Biochemistry, Microbiology and Biotechnology Kenyatta University Signature.....Date.....

# Prof. Walter Jaoko

Department of Medical Microbiology University of Nairobi Signature...... Date......

# Prof. Christine Sekadde-Kigondu

University of Nairobi

Signature..... Date.....

# DEDICATION

I dedicate this work to my wife Catherine W. Ireri, my daughters: Mercy Kendi and Favour Muthoni.

#### ACKNOWLEDGEMENTS

I acknowledge the Almighty God for his grace and strength in the entire work and I thank Him. I am indebted to my esteemed supervisors: Prof. Joseph J.N, Ngeranwa, Dr. David N, Mburu, Prof. Walter Jaoko and Prof. Christine Sekadde-Kigondu for the relentless guidance and overwhelming support they accorded me all through as I labored in this work and wish them God's blessings. I wish to deeply acknowledge the great and vital role played by Dr. Piero Ngugi of Department of Biochemistry and Biotechnology, Kenyatta University in guiding in the final corrections of this thesis; may God bless him. Moreover, I recognize Prof. Grace Kitonyi for the support and encouragement she constantly offered during the entire period as I labored; may God richly bless her. The Clinical and Laboratory staff at Kenyatta National Hospital Comprehensive Care Center (CCC) were so supportive and offered such a conducive working environment, I am grateful. I sincerely recognize the role played by Dr. Peter Wanzala of Kenya Medical Research Institute (KEMRI), Mr. Wycliff Ayieko of University of Nairobi (UoN) and Clayton S. Jisuvei of University of Nairobi (UoN) in statistical analysis. Mr. Peter Ngugi of Kenyatta National Hospital (KNH) and Mr. David Kibe of University of Nairobi (UoN) offered the highly valued assistance in laboratory analysis and I am thankful.

# **TABLE OF CONTENTS**

DECLARATIONi	i
DEDICATION	i
ACKNOWLEDGEMENTS	1
LIST OF TABLES	i
LIST OF FIGURES	ζ
ABBREVIATIONS AND ACRONYMS	i
ABSTRACTxii	i
CHAPTER ONE	L
INTRODUCTION	L
1.1 Background information	L
1.2 Statement of the problem and justification of the study	3
1.3 Significance of the study	1
1.4 Null Hypotheses	5
1.5 Objectives of the Study	5
1.5.1 General Objective	5
1.5.2 Specific Objectives	5
CHAPTER TWO	7
LITERATURE REVIEW	7
2.1 Global AIDS Burden	7
2.2 Pathogenesis of Human Immunodeficiency Virus (HIV)	3
2.3 Blood Cell Changes in HIV Infection	)
2.4 HIV-Associated Anaemia	3
2.5 Erythropoietin and Erythropoiesis	5
2.6 Erythropoietin and HIV Associated Anaemia15	5
2.7 Levels of Tumor Necrosis Factor-alpha in HIV16	5
2.8 Levels of Interlukin-6 in HIV	3
2.9 Levels of C - Reactive Protein (CRP) in HIV	)
2.10 Therapeutic Uses of Epo	)
2.11 Liver Function profiles in HIV infections	2
2.12 Kidney Function profiles in HIV-infections	5

CHAPTER THREE	29
MATERALS AND METHODS	29
3.1 Study site	29
3.2 Experimental design	29
3.3 Study population	29
3.4 Inclusion and exclusion criteria for HIV positive respondents	
3.4.1 Inclusion criteria	
3.4.2 Exclusion criteria	
3.5 Inclusion and exclusion criteria for HIV negative respondents	
3.5.1 Inclusion criteria	
3.5.2 Exclusion criteria	
3.6 Sample size calculation	31
3.8 Blood collection	
3.9 Experimental procedures	
3.9.1 Erythropoietin assay using a double-antibody sandwich ELISA	
3.9.2 CD4+ cell counts analysis	34
3.9.3 Haematological analysis	34
3.9.4 Peripheral blood film examination	35
3.9.5 C-Reactive Protein immunoturbidimetric test	35
3.9.6. Test for creatinine	35
3.9.7 Test for blood Urea	
3.9.8 Interleukin- 6 assay	
3.9. 9 Tumor necrosis factor-alpha assay	37
3.9.10 Assay for direct bilirubin	
3.9.11 Assay for total bilirubin	
3.9.12 Assay for L-gamma (γ)-glutamyl transferase (GT)	
3.9.13 Assay for alanine aminotransferase /Glutamyl pyruvate transferase (GPT)	
3.9.14 Assay for aspartate aminotransferase/Glutamate oxaloacetate transferase	
3.9.15 Determination of total protein	
3.9.16 Albumin assay	40
3.9.17 Alkaline phosphatase (AP) test	

3.10 Quality assurance	40
3.11 Data management and statistical analysis	41
3.11 Study Limitations	41
CHAPTER FOUR	43
RESULTS	43
4.1 Development of reference ranges for the HIV negative control population	43
4.1.1 Social demographics of the HIV negative control population	43
4.1.2 Reference ranges of blood parameters of the control population	44
4.2 Effects of HIV infection in female and male subjects on the Haematological	56
parameters	56
4.2.1 Social demographics of HIV seropositive population	56
4.3 Effects of HIV infection in male and female subjects on the levels of Biochemical	76
4.4 Effects of HIV infection in male and female subjects on the levels of Epo, TNF- $\alpha$ ,	89
IL-6 and CRP parameters	89
CHAPTER FIVE	95
DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS	95
5.1 DISCUSSION	95
5.2 CONCLUSIONS	103
5.3 RECOMMENDATIONS	107
5.3.1 Recommendation from the study	107
5.3.2 Recommendations for future study	108
REFERENCES	109
APPENDICES	131
Appendix 1: Kenyatta National Hospital /University of Nairobi (KNH/UoN) Ethics and	
Research committee	60
Appendix 2: Consent participants 18- 60 years	133
Appendix 3: Quality Control for CD4 Cell Counts	135
Appendix 4: Quality Control Haematological Parameters	136
Appendix 5: Quality Control for Biochemical parameters	137
Appendix 6: Tests the Normality of Referents' Parameters	138
Appendix 7: Tests for the Normality of HIV Positive Respondents' Parameters	141

# LIST OF TABLES

Table 4.1: Social demographic characteristics of the referents 44
Table 4.2: Means, standard deviations, medians, modes, 95% CI, 2.5 th -97.2 th percentiles
and p-values of female and male47
Table 4.3: Means, standard deviations, medians, modes, 95% CI, 2.5 th -97.2 th percentiles
and p-values of female and male Biochemical parameters
Table 4.4: Means medians, SD, modes, Ranges, 2.5 -97.5 percentiles Z-value, p-value of Epo
and Proinflammatory cytokines55
Table 4.5: Social demographic characteristics of the HIV- seropositive group
Table 4.6: Frequency of microcytic hypochromic anaemia in ART females      59
Table 4.7: Frequency of microcytic hypochromic anaemia in ARV- naive females
Table 4.8: Frequency of microcytic hypochromic anaemia in ART males    60
Table 4.9: Frequency of macrocytic anaemia in ART females    60
Table 4.10: Frequency of macrocytic anaemia in ARV-naïve females    61
Table 4.11: Frequency of macrocytic anaemia in ART males
Table 4.12: Frequency of macrocytic anaemia in ARV-naive males 62
Table 4.13: Frequency of parameters leukocytosis and thrombocytosis in ART females
Table 4.14: Frequency of parameters leukocytosis and thrombocytosis in ARV-naive
females65
Table 4.15: Frequency of parameters leukocytosis and thrombocytosis in ART males
Table 4.16: Frequency of, Leucopenia, Neutropenia lymphopenia, monocytopenia and
thrombocytopenia in ART Female66
Table 4.17: Frequency of lympopenia in ART males 67
Table 4.18 Referents compared Female ART baseline parameters    69
Table 4.19: Referents compared Female ARV-naive baseline parameters    69
Table 4.20: Referents compared with ART male baseline parameters
Table 4.21: Referents compared ARV- naïve male baseline parameters    70
Table 4.22: Differences in ART female haematological parameters during follow up
Table 4.23: Differences in ARV-naive female haematological parameters during follow up . 72
Table 4.24: Differences in ART male haematological parameters during follow up74
Table 4.25: Significant differences in haematological parameters between ART and

ARV-naïve females......75 Table 4.27: Within group changes in haematological parameters in ARV-naïve females........76 Table 4.30: Referents compared with ARV-naïve female baseline Biochemical parameters...78 Table 4.32 Referents compared with ARV-naïve male baseline Biochemical parameters .... 80 Table 4.34: Differences in ARV-naive female haematological parameters during Follow up ...84 Table 4.36: Differences in ARV-naive male haematological parameters during follow up ..... 86 Table 4.37: Differences in Biochemical parameters between ART and ARV- naive females. 87 Table 4.38: Differences in Biochemical parameters between ART and ARV- naïve males ...... 88 

 Table 4.49: Differences between ART and ARV-naïve females in cytokines
 93

# LIST OF FIGURES

Figure 4.1: Differences between female and male red blood cell mean values	.7
Figure 4.2: Differences between female and male haemoglobin mean values	.7
Figure 4.3: Differences between female and male WBC, N, L M and E mean values	-8
Figure 4.4: Differences between female and male CD4+ cells mean values	-8
Figure 4.5: Differences between female and male platelet mean values	.9
Figure 4.6; Differences between female and male AST, AIT and ALP Mean values	2
Figure 4.7; Differences between Female and male T.Bil and D.Bil mean values	2
Figure 4.8; Differences between female and male T>PRT and ALB mean values	3
Figure 4.9: Differences between female and male creatinine mean values	3
Figure 4.10: Normocyte (R) and a small mature	8
Figure 4.10: Microcytic hypochromic cells (M) Lymphocyte (R15	8
Figure 4.11: Rouleaux formation (R)5	8
Figure 4.12: Target cells (T)5	8
Figure 4.13: Round macrocyte (RM)5	8
Figure 4.14: 3-lobe neutrophil normal (N1)6	i4
Figure 4.15: 6 lobed neutrophil, hypersegmenteded (N2)6	i4
Figure 4.16: Eosinophil with 3 nulear lobe (E)6	i4
Figure 4.17: Hypersegmented eosinophil (E1)	i4
Figure 4.18: Norrmal platelet (p) and a giant platelet (P1)	<i>i</i> 4

# ABBREVIATIONS AND ACRONYMS

ACD	Anaemia of chronic disease
AIDS	Acquired Immune Deficiency Syndrome
ALP	Alkaline phosphatase
ALT	Alanine Aminotransferase
AMI	Acute myocardial infarction
ANOVA	Analysis of variance
ARV	Antiretroviral
AST	Aspartate aminotransferase
AZT	Azidodeoxythymidine
β	Beta
BCDF	B-cell differentiating factor
BFU-E	Burst forming unit – erythroid
BSF	B-cell stimulating factor
BUN	Blood urea nitrate
CCC	Comprehensive care clinic
CD	Cluster of differentiation
CDF	Cholinergic differentiation factor
CFU-G	Colony forming unit – granulocyte
CRP	C-reactive protein
CRT	Creatinine
CTL	Cytotoxic-T lymphocyte
D. Bill	Direct bilirubin
DNA	Deoxyribonucleic acid
Ε	Eosinophil
EDTA	Ethylene diamine tetra acetic acid
ELISA	Enzyme-linked immunosorbent assay
EPO	Erythropoietin
EpoR	Erythropoietin receptor
g/dl	Grams per deciliter
GM-CSF	Granulocyte macrophage-colony stimulating factor
G-CSF	Granulocyte-colony stimulating factor
GGT	Gamma glutamyl transferase
HAART	Highly active antiretroviral therapy
Hb	Haemoglobin
HbF	Fetal haemoglobin
HIF	Hypoxia-inducible factors
HGF	Hepatocyte growth factor
HIV	Human immune deficiency virus

HPGF	Hybridoma/placytoma growth factor
IL	Interleukin
IU/L	International units per liter
KNH	Kenyatta National Hospital
L	Lymphocyte
LFT	Liver function test
Μ	Monocyte
MCH	Mean cell haemoglobin
MCV	Mean cell volume
MCHC	Mean cell haemoglobin concentration
M-CSF	Macrophage colony stimulating factor
Mg/dl	Milligram per deciliter
MGI-2	Macrophage growth inducer
Mm <sup>3</sup>	Millimeter cubed
mRNA	messenger RNA
Ν	Neutrophil
Nm	Nanometer
OIs	Opportunistic infections
Р	Platelet
PCV	Packed cell volume
PI	Principal investigator
RBC	Red blood cell
R-HuEpo	Recombinant human erythropoietin
RNA	Ribonucleic acid
SIE	Serum immunoreactive erythropoietin
SPSS	statistical package for the social sciences
T.Bil	Total bilirubin
TNF-α	Tumor necrosis factor-alpha
TNF-R	Tumor necrosis factor receptor
U/E/C	Urea/ electrolyte/creatinine
U/I	Units per liter
UREA	Urea
μl	Microliter
µmoles./l	Micromoles per liter
WBC	White blood cells

There were between 31.6 million and 44.5 million HIV infected people globally by the year 2019, 70% of them in sub-Saharan Africa and Kenya harbored 1.6 million. Anaemia is the most common complications in HIV. Erythropoietin, and the proinflammatory cytokines (TNF-a, IL-6 and CRP) are markers of anaemia. The liver produce CRP and the kidney Epo. Changes in blood cell morphologies, levels of haematological, biochemical parameters, Epo, the proinflammatory cytokines in HIV have not been determined among Kenyans. This study aims at determining changes in the levels of these parameters in HIV infected patients. A total of 184 HIV infected adults seen at KNH and 202 blood donors formed the basis of this study. The HIV infected were grouped into: CD4<200 -, 200-499- and  $\geq$ 500 cells/mm<sup>3</sup> and followed for 6 months. Blood samples for assessing levels of the parameters were taken. SPSS version 21 was used to analyze the data, Shapiro-Wilk test determined the normality of the data. Bootstrap parametric and non-parametric methods were used to raise the power of the low sample sizes of referents. Data was described by medians, 2.5-97.5<sup>th</sup> centiles, means and standard deviations. Wilcoxon rank-sum test compared independent variables and Kruskal Wallis test compared values between CD4 groups. Among the referents, significantly higher mean values in females than in males were demonstrated in: WBC (p=0.002), N (p =0.004), L (p=0.001), CD4+ cells (p<0.001) and P (p<0.001); while lower mean values were demonstrated in RBC (p<0.001), HB (p<0.001) and E (p=0.004). In Biochemical values, females demonstrated significantly higher mean values than males in ALB (p<0.001), but lower in AST (p=0.001), ALT (p<0.001), ALP (p< 0.001, T.Bil (p=0.004), D.Bil (p<0.001), T.PRT (p<0.001) and CRT (p=0.001). In cytokines significantly higher median values in females were demonstrated in CRP (p<0.00I) and lower in: Epo (p<0.020), TNF-a (p<0.001) and IL- 6 (p=0.016). During follow up, microcytic hypochromic anaemia coupled with rouleaux of up to 15.4% and 15.4% were demonstrated in ART and ARV - naïve females and up to 50% in ART males. ART females demonstrated significantly higher RBC and HB mean values than ARV females. Macrocytic anaemias together with targets of up to 28.2 %, 50% in ART and ARV-naïve females respectively and up to 25% in both ART and ARV-naïve males were demonstrated. Neutrophilia together with hypersegmentation of up to 15%, 25% in ART and ARV-naïve females respectively and 25% in males; eosinophilia of up to 11.1% in ART females thrombocytosis of up 11.1% in ART females and 25% in males in  $CD4 \ge 500$  group; Lymphopenia of up to 33% in ART females and 50% in ARV males decreasing with increases in CD4 counts were demonstrated. In all the CD4 groups of all the respondents increases in AST, ALT, and GGT were demonstrated. Increases in ALB and ALP were demonstrated in ARV-naïve females in CD4 < 500 group. Decreases in T.PRT and increases ALB were demonstrated in females. Demonstrated were baseline increases in Epo, TNF- $\alpha$  and decreases in CRP and IL-6 and persistent increase in TNF- $\alpha$  in ART females. Recommendations were: development of gender-based reference ranges for: routine Haematological and Biochemical parameters, proinflammatory cytokines and use of L counts in HIV management in resource-limited setting.

#### **CHAPTER ONE**

# **INTRODUCTION**

#### **1.1 Background information**

Haematological complications among human immunodeficiency virus (HIV) patients generally present as pancytopenia (Ogba et al., 2013; Tamir et al., 2019). The incidence and severity of pancytopenia correlate with the stage of the disease. The most frequent cytopenia, is anaemia occurring in 70 - 80% globally and in 80-90 % of HIV infected patients in the developing countries (Semba and Gray, 2001; Kasthuri et al., 2006; Yesuf et al., 2019). In the laboratory anaemia is diagnosed using total blood counts (TBC) and blood film evaluation to define its morphological characteristics. World Health Organization (WHO), defines anaemia as haemoglobin (Hb) levels below 12 g/dl and 13 g/dl in females and males, respectively (Beutler and Waalen, 2006; WHO 2011). The main contributors to anaemia development in HIV infection include myelosupressive drugs especially zidovudine (AZT) (Meidani et al., 2012; Ikunaiye et al., 2018), HIV infection alone without other complicating illness, soluble factors in the serum of HIV infected persons that may inhibit haemopoiesis (Ferede and Wondimeneh, 2013; Zerihun et al., 2019), haemolysis due to antierythrocyte antibodies, infection-related gastrointestinal bleeding (Adewumi et al., 2014; Abdelfatah and Tuttle, 2017) and hypogonadism in men (Carrero et al., 2012; Blick et al., 2013; Gomes et al., 2017).

The most common complication in AIDS that is associated with the disease progression and poor clinical outcomes is anaemia. For effective management of HIV disease, details on the pathophysiology of HIV-associated anaemia need to be well elucidated. Changes in the levels of haematological parameters in HIV infected patients monitored over time as envisaged in this study would offer valuable information towards this goal. Most of such information has been derived from cross sectional studies (Obirikorang and Yeboan, 2009; Parinitha and Kulkarni, 2012; Mathews *et al.* 2013; Panwar *et al.* 2016; Kasthuri, *et al.*, 2019).

Concurrently with anaemia, inflammation has been reported to complicate HIV disease prognosis. Some of the proinflammatory cytokines reported to be associated with anaemia include TNF-a, IL-6 and CRP. Erythropoietin (Epo) hormone promotes erythropoiesis and serves in replenishing the blood cells (De Araujo *et al.*, 2014; Beverborg *et al.*, 2015). The dynamics of Epo levels during cytopenic and inflammatory episodes raises immense concern. Inclusion of information on levels of Epo, TNF- $\alpha$ , IL-6 and CRP during prospective monitoring of the levels of haematological parameters would thus be key in establishing the mechanism through which HIV-associated anaemia develop. Erythropoietin is produced in the kidney; while CRP is synthesized in the liver. Functional status of the kidney and the liver will determines serum levels of Epo and CRP respectively. Kidney function is assessed by doing creatinine and urea assays among other tests. Liver function status is assessed in the laboratory by determining blood levels of liver enzymes such as Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyltransferase (GGT), and alkaline phosphatase (ALP) including bilirubin, total protein, and albumin levels. Tumor necrosis factor-alpha is produced primarily by macrophages; while IL-6 is produced by lymphocytes and monocytes. These cells can be quantified in TBC save for macrophages which are transformed monocytes and located in tissues other than the blood.

There are numerous reports on significant differences in clinical laboratory reference intervals within African populations and between Africans and Caucasians. These variations have been attributed to differences in: race, geographical locations, sex, altitude, climate, diet, and environment (Ngovi *et al.*, 2009; Mine *et al.*, 2011; Subbiah, 2017). To reduce the risk of influence by these peculiar differences on the reference ranges, it is critical to use reference ranges derived from populations with similar social-demographic characteristics as the patients. In this study, gender-based reference ranges used for interpreting results for changes in: haematological, biochemical, Epo and the pro-inflammatory cytokine levels in HIV positive respodents were derived from HIV- negative adults recruited in the same study setting as the study subjects.

# 1.2 Statement of the problem and justification of the study

The world's AIDS burden stood at 38.8 million by 2015 following a decline in annual incidence of 3.3 million new infections in 1997 to a relatively constant incidence of 2.9 million. At the same time AIDS-related deaths declined from 1.8 million to 1.2 million annually. Sub-Saharan Africa bore 70% of this burden with Kenya being home to 1.5 million HIV-positive adults. This decline has been credited to the improvement in ART and prevention of the disease transmission from infected mothers to their children (Kyi and Sidibem, 2012; Wang, 2016; Mutabazi *et al.*, 2017). Hitherto, cases of HIV infection have been increasing considerably with the global burden now standing at between 31.6 million - 44.5 million HIV infected people and Kenya harboring 1.6 million with an estimated prevalence of 4.9% (NASCOP, 2018; UNAIDS Strategy 2016–2021). The main bottle neck faced by ART which is the main intervention tool is the complications posed

by HIV-associated anaemia. It is, therefore, important to obtain detailed information on the pathophysiology of the anaemia as this would be important in improving therapeutic and management options (Belperio and Rhew, 2004; Reedit and Berliner, 2013; .Huibers *et al.*, 2020). Available information towards this goal has been largely derived from crosssectional studies using reference ranges from populations of Caucasian descent. (Aslinia *et al.*, 2006; Meidani *et al.*, 2012; Panwar *et al.*, 2016; Kasthuri, *et al.*, 2019).

This study, therefore, monitored changes in: Haematological and Biochemical parameters, Epo and pro-inflammatory cytokines in active HIV disease over time using reference ranges derived from HIV negative blood donors with similar demographics characteristics with the patients in order to explain further the mechanisms associated with anaemia development. The study was conducted at KNH located in Nairobi County with HIV prevalence 6.5%, similar to that of the Nation (National AIDS Control Council, 2016) and where ART programme was first well established under the United States President's Emergency Plan for AIDS Relief through the University of Nairobi's AIDS Care and Treatment Services (2003–2010) and the Centers of Excellence Project (2010–2016) (Mecha, *et al.*, 2016).

# **1.3 Significance of the study**

This study monitored changes in Haematological and Biochemical parameters including erythropoietin and some pro-inflammatory cytokines in HIV positive ART and ARVnaïve patients at different stages of the disease over a six months period. Interpretation of these changes was done using reference ranges derived from referents with similar social demographic characteristics as the patents. The findings described the various types of

4

anaemia morphologically and related this anaemia to changes in biochemical parameters and Epo together with the cytokines.

The results will be utilized by health care givers managing HIV- associated anaemia as managerial options are determined by the type of anaemia. For example, microcytic hypochromic anaemia may require iron concentrates; while megaloblastic may require vitamin  $B_{12}$  or folate administration and still normocytic normochromic may require transfusion with packed cells. Moreover, further studies in the same setting will utilize the obtained results as a fundamental foundation to build on. The determined reference ranges can be utilized in both research and diagnostic services.

# **1.4 Null Hypotheses**

- i. The established reference intervals for haematological, biochemical, Epo and proinflammatory cytokines do not significantly differ between females and males.
- ii. HIV infection does not affect blood cells morphologies, haematological and biochemical parameters, and biomarkers of anaemia in males and females at different levels of CD4 counts.

# 1.5 Objectives of the Study

### **1.5.1 General Objective**

To determine changes in Haematological and Biochemical parameters and Erythropoietin and pro-inflammatory cytokine in HIV infected patients at Kenyatta National Hospital.

# **1.5.2 Specific Objectives**

- To establish gender-specific 95% reference intervals for selected haematological and biochemical parameters, and Epo, II-6, TNF-α and CRP values in healthy adults at Nairobi county, Kenya.
- ii. To determine the effects of HIV infection in female and male subjects on the blood cell morphology and haematological parameters at different levels of CD4 counts.
- iii. To determine the effects of HIV infection in female and male subjects on the levels of biochemical parameters at different levels of CD4 counts.
- iv. To determine the effects of HIV infection in female and male subjects on the levels of erythropoietin, tumor necrosis factor-  $\alpha$ , interleukin-6 and C-reactive protein, in the studied CD4 groups

#### **CHAPTER TWO**

# LITERATURE REVIEW

### 2.1 Global AIDS Burden

It is estimated that world-wide HIV incidence reached maximum in 1997, at 3.3 million new infections. A relatively constant annual incidence of 2.9 million per year was observed since 2005, following a period of decrease between 1997 and 2005. The number of HIV infected people globally reached 38.8 million by 2015. Conversely, there has been steady decline in HIV-related death rates from 1.8 million in 2005 to 1.2 million deaths in 2015. This has been attributed to majorly the improved antiretroviral therapy and prevention of transmission of AIDS from infected mothers to their children in the past two decades. The proportion of the people living with HIV and receiving ART between 2005 and 2015 increased from 6.4 % to 38.6% (Kyi and Sidibem, 2012; Wang, 2016). According to United Nations 2016-2021 AIDS Strategy, there were between 31.6 million and 44.5 million HIV infected people globally of whom about 22 million had not accessed ARVs by the year 2019 (UN AIDS Strategy 2016–2021).

Furthermore, more than 70% of world's HIV/AIDS burden is borne by sub-Saharan Africa. There were 1.8 million new HIV infections in 2015, with large proportion in Western, Southern and Eastern sub-Saharan Africa. Prevalence and mortality have also been greatest in sub-Saharan Africa. Infection rates varied tremendously across countries with Southern Africa having the highest rate of infection, where more than 1 % of the population was getting infected per year in Botswana, Lesotho and Swaziland. Kenya was among nine countries (Central African Republic, Cameroon, Equatorial Guinea,

Mozambique, Malawi, Tanzania, Uganda and Zambia) in sub-Saharan Africa which had a prevalence of more than 2.5% of the entire population (Wang, 2016; Kharsany and Karim, 2016). By the year 2004, in Kenya, only about 432,621, people out of the 1.5 million HIV infected people (61%) were receiving ARVs. The majority of these were managed within the private sector, non-governmental organizations and Faith Based Health facilities (Speight and Kenneth, 2004). Since then, however, ART coverage was up-scaled and according to the Kenya National AIDS and sexually transmitted infection (STI) programme report of 2016 the coverage varies from county to county with Kericho being least covered (67%) and Marsabit being most covered (125%). Nairobi County is reported to have HIV prevalence that is comparable to national prevalence at 6.1% (National AIDS Control Council, 2016). In June 2016 the World Health Organization (WHO) updated the guidelines on the use of ARVs for treating and preventing HIV infection in which all people living with HIV were eligible for ART (WHO, 2016).

#### 2.2 Pathogenesis of Human Immunodeficiency Virus (HIV)

Human Immunodeficiency Virus is mainly transmitted through blood and genital fluids and from infected mothers to newborns. Once inoculated, the virus primarily infects cells bearing CD4+ receptors on their membranes. Such cells include: T-helper cells, monocytes, macrophages, glial cells, chromaffin cells and Langerhans' cells. There are two glycoprotein (gp) molecules on the HIV 1 envelope: gp 120 and gp 41. The virus enter the host cell by attaching its gp 120 to the CD4+ cell receptor on the cell membrane. The interaction between the virus and the chemokine co-receptors CCR5 and CXCR4 results in irreversible configurational change with subsequent pore formation enabling the fusion process to occur. In the host cell as well as in vitro the tropism of the virus is defined by the distribution of the co-receptors on activated CD4+ T-lymphocytes (Plat *et al.*, 2005). Once in the cell the virus transcribes its RNA to host cell proviral DNA by using its reverse transcriptase enzyme. The cell is then directed to produce more HIV virions by the proviral DNA. The virions are then released. HIV depletes CD4+ T-lymphocytes through mechanisms that are not clearly understood. These mechanisms may include destruction of the infected cells by: the viral replication, anti-HIV immune response, or by homing of the resting CD4 lymphocytes from the blood stream to the peripheral lymph nodes and axial bone marrow after coming into contact with HIV (Cloyd *et al.*, 2001). From primary infection and throughout the entire course of infection, the loss of CD4+ cells is continuous (Levy, 1993; Weber, 2001). When the virus interacts with different cells in the body and escape the host immune response against it AIDS results (Weber, 2001).

Symptoms of seroconversion illness that may occur during primary infection include: rash, painful lymphadenopathy, arthropathy and fever. This phase of the infection may be asymptomatic although the virus is actively replicating in the lymph nodes and blood stream. Possibly due to primary immune response to HIV the viraemic peak resolves spontaneously within 2 - 4 weeks. During primary infection, high viral load is associated with decline in CD4<sup>+</sup>T-cell counts in peripheral blood. If the decline of CD4+ T-cells goes below 200 x  $10^9$ /l, it may lead to immunosuppression which may in turn results in cases of opportunistic infections such as Pneumocystis carinii pneumonia and oesophageal candidiasis. As the viral load reduces, the CD4+ T-cell counts return towards baseline levels, (Levy, 1993; Naif, 2013).

During the asymptomatic phase of HIV infection, there is a slow decline in CD4+ T-cell counts and the HIV 1 RNA levels range from < 50 copies RNA / ml, to > 1,000,000 copies RNA /ml depending on individuals. The decline in CD4+ T-cells is as a result of either cell death or of reduced production, or both following viral replication. There is active specific humoral and cellular immune response during this phase. In the late stage of the infection when CD4<sup>+</sup> T-cell counts fall below 200 x  $10^9$ /l, the HIV specific cellular immunity is exhausted (Weber, 2001; Paiardini *et al.*, 2013). Due to the depleted immunity opportunistic infections set in. These include: malignant neoplasms (like Kaposis sarcoma), protozoan (like pneumocystis carinii), fungal (like Candidiasis), bacterial (like tuberculosis), viral (like Cytomegalovirus) and the list goes on. Opportunistic infections are the major complications in HIV infection and cause high mortality rates if corrective measures are not instituted. It is pertinent noting that changes in CD4+ T-lymphocytes levels in the peripheral is a good predictor of HIV disease severity (Vijayan *et al.*, 2017).

### **2.3 Blood Cell Changes in HIV Infection**

A major morphologic finding on the peripheral blood smear from an HIV infected individual is marked anisocytosis and poikilcytosis, resulting in an increased red cell distribution width (RDW). Rouleaux formation, leucopenia with hyposegmented granulocytes and vacuolated monocytes have also been observed in HIV infections (Ogba *et al.*, 2013; Jacob, 2016). Microcytosis is uncommon in the absence of bleeding and macrocytosis is infrequent except in patients receiving zidovudine therapy. Development of microcytic hypochromic cells is a manifestation of iron deficiency. Panwar *et al.* (2016) demonstrated that iron deficiency significantly (p = 0.022) contributed 42.85% of microcytic hypochromic anaemia among HIV infected Indians in a cross-section study. Iron deficiency results in inadequate oxygen delivery due to reduced levels of haemoglobin. Iron deficiency on brain neurotransmitters result in neurological and cognitive abnormalities. Physical activity is affected by defective oxidative metabolism in the skeletal muscle. This would consequently affect the productivity of AIDS victims which translates to decreased economic performance of a country (Treacy *et al.*, 1987; Zizola and Schulze, 2013).

The microcytosis described by Panwar *et al.* (2016) need to be quantified and the levels monitored prospectively in different stages of AIDS and correlated with CD4+ T-lymphocytes. This would show the relationship between the disease severity and the level of microcytosis. Any established relationship would facilitate further investigations in ruling out nutritive iron deficiency against defective iron metabolism. Whereas iron deficiency is correctable using iron concentrates, metabolic defects cannot.

Presence of red cells that are larger than normal (macrocytes) is mainly indicative of vitamin  $B_{12}$  and folate deficiency. The study by Panwar *et al.* (2016) showed that significantly macrocytosis was due to vitamin  $B_{12}$  and folate deficiencies (47.05%, p = 0.003 and 35.29%, p = 0.012, respectively). Macrocytosis due to these deficiencies is due to megaloblastic changes characterized by oval macrocytes in peripheral blood (Aslinia *et al.*, 2006). During haemopoiesis, DNA replication occurs. An essential step in DNA replication is the conversion of deoxyuridine to thymidine using methyl tetrahydrofolate. Folate or vitamin B <sub>12</sub> is required in the formation of this folate. Therefore deficiency of either of these vitamins result in either short supply of thymidine or misincorporation of deoxyuridine into DNA in place of thymidine. This results in: defective DNA replication,

breakage of secondary strands and deranged growth and maturation of rapidly diving cells including haemopoietic cells. As a result there is development dysynchrony between the cell nucleus and cytoplasm. The abnormal development of the former and the apparent normal development of the later results in macrocytic erythrocytes.

Other causes of macrocytosis (other than folate or vitamin  $B_{12}$  deficiencies) include: alcoholism, chronic renal failure, drugs, myelodysplastic syndrome, aplastic anaemia, liver disease and non-pathological macrocytosis in pregnancy (Veda, 2012; Devi *et al.*, 2016). Macrocytes resulting from non-megaloblastic changes are characterized by presence of round macrocytes seen in peripheral blood. These macrocytes emanate from red cell membrane excesses due to excess lipid deposition on the cell membrane (Veda, 2012). Megaloblastic macrocytosis is correctable by administering folate or vitamin  $B_{12}$ concentrates, while non megaloblastic macrocytosis is not. Macrocytosis described in HIV infection need to be described into either megaloblastic or non-megaloblastic etiologies. This would facilitate in instituting the correct remedial measures.by the health care givers.

Deficiencies of cobalamin and /or folate also produce hypersegmentation of polymorphonuclear leucocytes. Red blood cells, and neutrophils may also be decreased in numbers. Rouleaux formation has been noted in a minority of peripheral smears in HIV infected patients and probably arise from polyclonal hypergammaglobulinaemia (Mazza, 2001). The blood cell changes have mainly been described in cross section studies. Quantification and monitoring of the changes in varying levels of HIV severity prospectively would be essential in monitoring the process of recovery from anaemia following treatment. Peripheral blood film is an invaluable routine laboratory test that is

used for screening, diagnosing and monitoring of disease progression and therapeutic response. Its application in hematology is still held in high esteem despite advances in hematology automation and application of molecular techniques (Adewoyin and Nwogoh, 2014).

#### 2.4 HIV-Associated Anaemia

When haemoglobin levels fall below 12 g/dl in females and below 13 g/dl in males anaemia is said to occur (Beutler and Waken, 2006). It is a frequent complication in advanced HIV infection. As many as 70 - 80% of HIV infected patients develop anaemia in the course of infection. Prevalence level of the anaemia is even higher, in developing countries where prevalence levels of between 80 - 90% have been reported. This has been attributed to largely unavailability of antiretroviral therapy (Semba and Gray, 2001). Among HIV seropositive patients receiving myelosuppressive therapies anaemia has been demonstrated in: 8% of those with asymptomatic HIV, 20% of those with asymptomatic middle - stage of HIV and 70% of those with Centers for Disease Control-defined AIDS (Meidani et al., 2012). In this study the most common type of anaemia overall was normocytic with decreasing reticulocyte count. This was a cross-section study and so could not define trends of the reported levels of anaemia over time. In another study involving 710 HIV infected antiretroviral naïve and 226 HIV negative Rwandese women it was observed that anaemia was higher in HIV positive group (20.5% vs 6.3%, p < 0.002). This anaemia increased with lower CD4 counts:  $\geq$  350 (7.6%), 200 – 349 (16%) and < 200 cells/mm<sup>3</sup> (32.2%) (Munyazesa *et al.*, 2012). The study involved only female participants and so the results cannot be applied to males who are known to have significantly different haematological values from females (Lugada et al., 2004; Korum et al., 2007; wakeman *et al.*, 2007; Saathoff *et al.*, 2008; Menard *et al.*, 2009). This was a cross - sectional study and could not capture the trends of changes in anemia over time.

Infection of the marrow progenitor cells by HIV result in anaemia and other haematologic abnormalities. Haemopoiesis may be suppressed by soluble factors associated with HIV infection (Kreuzer and Rockstroh, 1997; Ferede and Wondimeneh, 2013). It has been reported that 34% of patients with advanced HIV disease treated with Zidovudine (AZT) in phase II clinical trials developed anaemia that was accompanied with progressive rise in erythrocytes mean cell volume. This suggests that AZT therapy may be a common cause of anaemia in HIV infection (Richman et al., 1987; Mugisha et al., 2012). Antierythrocyte antibodies have also been cited as possible causes of anaemia in HIV infection, although HIV-related positive direct antiglobulin test in the hemolysis is rare. Gastrointestinal bleeding caused by opportunistic infections such as cytomegalovirus, colitis, and malignancies result in anaemia (Tsiako et al., 2011; Adewumi et al., 2014). In men, hypogonadism has been pointed as a possible contributor to anaemia development in HIVinfection (Carrero et al., 2012; Ruchira et al., 2015). The following levels of anaemia have been demonstrated in 250 HIV ARV-naïve patients: normocytic normochromic (40.4%), dimorphic (18.8%) and microcytic hypochromic (7.2%). Conversely, in HIV ART patients anaemia was distributed as follows: normochromic (63.88%), microcytic hypochromic (19.44%), macrocytic (2.8%) and dimorphic (13.88%) (Parinitha and Kulkarni, 2012). Moreover, macrocytic anaemia of 34.62% has been demonstrated in 187 HIV AZT – treated Indians (Mathews *et al.*, 2013). Although these studies have given the levels and the morphological types of anaemia in HIV infection, these were cross sectional in nature and so could not provide the trend of the anaemia over time.

#### 2.5 Erythropoietin and Erythropoiesis

Erythropoietin (Epo) has a molecular mass of 39 KDa comprising of 165 amino acids and four carbohydrate groups. This glycoprotein hormone is produced by the peritubular interstitial fibroblasts in the cortex of the kidney and its production is induced by hypoxia and suppressed by hyperoxemia (Haase, 2010; Debevec *et al.*, 2012). Epo has a half-life of 6 - 9 hours and under normal conditions is maintained at approximately 10-20U/L in plasma (Wide and Bengtsson, 1990; Lee *et al.*, 2006; Grote *et al.*, 2015). High levels of Epo may hasten the transition from burst forming unit-erythroid (BFU-E) to Hb - synthesizing cell. Epo regulates red blood cell production and also promotes their survival by protecting them from apoptosis Indeed, Epo is an obligatory factor for erythroid development (Amanzada *et al.*, 2014; Beverborg *et al.*, 2015).

#### 2.6 Erythropoietin and HIV Associated Anaemia

During established HIV infection anaemia is associated with faster development of acquired immunodeficiency virus infection and death (Gatukui *et al.*, 2014). HIV infection suppresses bone marrow resulting in anaemia. Endogenous anti-Epo antibodies have also been associated with HIV-related anaemia (Tsiakalos *et al.*, 2010). HIV may also infect early erythrocytes in the marrow and reduce the lifespan of mature erythrocytes. Anaemia also occurs in HIV infected people who develop opportunistic infections. Anaemic AIDS patients receiving zidovudine have been shown to have serum Epo levels ranging from normal to markedly elevated levels, 9 - 3390 U/L (Rarick *et al.*, 1992; Carol and Ersieve, 1988; Artune and Risler, 2007). Direct correlation between Hb levels and the numbers of CD4+ lymphocytes in established HIV infection has been established (Saverino *et al.*, 1999; Obirikorang and Yeboan, 2009).

A cross-sectional study done on 200 HIV infected Rwandese women showed that anaemia levels increase with HIV - stage and decrease on HAART use and that treatment with zidovudine, opportunistic infections and low CD4+ counts are risk factors for anaemia development. However, the study, did not determine serum levels of the cytokines that influence the Epo production. The study also recommended further studies on long-term follow-up on the Hb changes in a similar study setting (Masaisa *et al.*, 2011; Dusingize *et al.*, 2015). In addition, a study involving 228 HIV infected Ghanaians divided into 3 CD4+ groups:  $\geq$  500-, 200 – 499- and < 200 cells/mm<sup>3</sup> together with 100 sex and age-matched seronegative controls, a significant correlation between Hb and CD4+ cells was demonstrated. In this study serum levels of the cytokines associated with anaemia in HIV were not determined (Obirikorang and Yeboan, 2009). Levels of erythropoietin, interleukin-6 and tumor necrosis factor- $\alpha$  in HIV associated anaemia will be important in predicting the trend and severity of anaemia. However, Obirikorang and Yeboan, did not incorporated erythropoietin, IL-6 and TNF- $\alpha$  assays in their study.

# 2.7 Levels of Tumor Necrosis Factor-alpha in HIV

The cytokine, TNF- $\alpha$  has a molecular weight of 17 kDa and contains 165 amino acid residues. It is synthesized as a type II transmembrane protein with a receptor binding motif located at the C-terminus. The cytokine, TNF- $\alpha$  is also referred to as lymphotoxin B and cachectin. This cytokine is secreted by cells such as macrophages, monocytes, neutrophils, T-cells, natural killer cells, CD4+ cells and CD8+. Its secretion is stimulated by: bacterial lipopolysaccharides, interferons, interleukin – 2 and granulocyte macrophage - colony stimulating factor. Other cells that secrete this cytokine include: stimulated neutrophils and some transformed microglial cells, smooth muscle cells and fibroblasts. The cytokine

is also found in human milk. Some of the properties of this cytokine include stimulatory and inhibitory processes and self-regulatory properties. Decrease in this cytokine levels enhances replacement of injured and senescent tissue by stimulating fibroblasts growth. Blood levels of the cytokine, TNF- $\alpha$  in health are 1.2-15.3 pg. /ml (Quest Diagnostics, 2013).

It has been reported that high levels of tumor necrosis factor- $\alpha$  and increased risk of mortality are associated (Popa et al., 2007). Sustained increase in this cytokine levels result in cachexia, net catabolism, weight loss and anaemia. These disorders are commonly associated with cancers and AIDS. High levels of this cytokine have an association with anaemia in HIV (Kalyani and Jamil, 2015). High levels of TNF- $\alpha$  and IL-1 $\beta$  may be inhibitory to Epo production. In addition, TNF- $\alpha$  is suppressive to bone marrow (Ellaurie and Rubinstein, 1995; Sade-Feldman et al., 2013) and promote apoptosis of erythroid precursor cells (Kreuzer and Rockstroh, 1997; Morceau et al., 2009). TNF-α interacts with IL-6 synergistically in inflammation and lymphocyte growth (Hoffbrand *et al.*, 2005; De Simone *et al.*, 2015). Production of TNF- $\alpha$  primarily occurs in peripheral cells including monocytes or macrophages (Beutler and Cerami, 1989). In view of this the effect of blood cell cytopenias observed in HIV (Kyeyune et al., 2014) on the changes in the levels of TNF- $\alpha$  have not been clearly explained. Monitoring prospective changes in both the levels of TNF- $\alpha$  and blood cell counts during active HIV infection will be a pointer to the trends of TNF- $\alpha$  levels in HIV associated anaemia.

Interleukin-6 has a molecular mass of 21-28 kDa depending on glycosylation. This pleiotropic cytokine is involved in immune response, inflammation and hematopoiesis. Its production occurs in cells such as monocytes, fibroblasts, endothelial cells and keratinocytes following stimulation by: TNF- $\alpha$ , lipopolysaccharides and IL-1 (Breen et al., 1990; Duque and Descoteaux, 2014). Some of its synonyms include: hybridoma/placytoma growth factor, macrophage granulocyte inducer-2, interferon-b2, hybridoma growth factor, cytotoxic T-lymphocyte-differentiating factor, cholinergic differentiation factor, and hepatocyte growth factor. Its level in blood in health is 0 - 14pg./ml. Production of IL-6 is regulated by glucocorticoids, catecholamines, and secondary sex steroids (Schuett et al., 2009; Quintanar and Guzmán-Soto, 2013). Increases in IL-6 levels are associated with increased risks of heart attack. High levels of IL-6 are observed in conditions such as sepsis, autoimmune diseases, lymphoma, AIDS, alcoholic liver disease, tumor development, Alzheimer's disease, and in transplantation rejection (Ridker et al., 2000; Luna et al., 2014). This pleiotropic cytokine stimulates the hepatic synthesis of hepcidin and this effectively diverts iron into iron stores resulting in iron-restricted erythropoiesis and anaemia. Interleukin-6 plays an essential role in the differentiation of activated B cells to immunoglobulin-secreting cells, modulation of bone resorption and is a major effector of inflammatory joint destruction in rheumatoid arthritis and in development of inflammation-associated carcinogenesis. Polyclonal B cell activation is commonly observed in HIV-infection (Breen et al., 1990; Moir and Fauci, 2009). Interleukin-6 has also inflammatory activities, induces acute phase reactions and influences T and B cells activities (Hoffbrand et al., 2005; Srirangan and Choy, 2010). Autoantibodies with high affininity to IL-6 have been reported in some blood donors (Ridker *et al.*, 2000; Fosgerau *et al.*, 2009). Levels of IL-6 increase in HIV infection (Henrik *et al.*, 1996). Nonetheless, little is known about the effect of HIV severity on the levels of IL-6. Monitoring levels of IL-6 in varying stages of HIV disease prospectively will provide information on HIV-associated pathologies like inflammatory reactions that are triggered by IL-6.

#### 2.9 Levels of C - Reactive Protein (CRP) in HIV

The site of CRP synthesis is the liver. In health, there are only trace amounts of CRP in serum, usually  $\leq 10$ mg/l increasing with age, late pregnancy, mild inflammation viral and bacterial infections. Marked increases in CRP levels occur following inflammatory stimuli. Measurements of CRP are useful in determining disease progress or the effectiveness of treatment (Brian and Olshaker, 1999; Jain *et al.*, 2011). In HIV infection, CRP levels in blood increase with decrease in CD4+ counts. In HIV infection, CRP levels can be used as a predictor of the disease progress with lower levels predicting longer survival of the infected individuals (Fieldman *et al.*, 2003; Drain *et al.*, 2007; Lungford *et al.*, 2007). The CRP production is mainly affected by liver failure (Pepys and Hirschfield, 2003; Chen *et al.*, 2015). A prospective case - cohort study involving 236 cases and 214 controls demonstrated that elevated CRP levels in HIV-associated anaemia can increase clinical failure after therapy with ARVs by 6-fold (Redd *et al.*, 2010; Shivakoti *et al.* 2015).

Although inflammation and anaemia have been reported to complicate HIV disease prognosis, changes in the levels of Epo and the cytokines associated with anaemia have not been done in different levels and types of anaemia in Kenya. This would provide information on the mechanism of anaemia development and facilitate in the managerial interventions.

#### 2.10 Therapeutic Uses of Epo

Epo hormone is the main treatment option for several types of anaemia including those related to cancer. The recombinant HuEpo (r-HuEpo), produced by a recombinant DNA technology in mammalian cell culture is used as a therapeutic agent. Examples of recombinant HuEpo products include: epoietin- $\alpha$ , - $\beta$ , epoietin- $\omega$ , epoietin- $\delta$  (Dynepo) and Darbepoietin- $\alpha$  (Aranesp) (Lin *et al.*, 1985; Egrie and Browne, 2001; Elliott *et al.*, 2003; Spinowitz and Pratt, 2006; Kiss *et al.*, 2010; Jelkmann, 2013; Henry, 2016).

Recombinant human Epo was initially approved for the treatment of anaemia arising from deficiency of endogenous Epo following chronic renal failure. However, it has now acquired widespread clinical use for more than fifteen years. It is important to note that renal failure accounts for approximately 2-3% of medical admissions in tropical countries (Naicker 2003; Legbo, 2016). Other clinical settings where the use of r-HuEpo has been approved include HIV, cancer, myelodysplastic syndromes, bone marrow transplantation hepatitis C (Winearls et al., 1986; Eschbach et al., 1987; Henry et al., 2004; Delanghe et al., 2008) and surgery (Stovall, 2001; de Araújo Azi et al., 2014). In patients with multiple myeloma receiving r-HuEpo for anaemia treatment, antineoplastic and immunomodulatory functions of Epo were observed (Mittelman et al., 1997; Rocchetta et al., 2011). Treatment with Epo also cause decreases in serum levels of interleukin-6 and normalizes the CD4:CD8 T-lymphocyte ratios (Prutchi-Sagir et al., 2006).

Anaemia in cancer patients in presence of increased levels of serum-Epo has been associated with increased levels of TNF- $\alpha$  and chemotherapy especially with platinum analogues which partially impair Epo response. So for cancer patients receiving chemotherapy early initiation of Epo treatment is recommended (Hoffbrand *et al.*, 2005; Grossi *et al.*, 2007). Treatment with Epo has been used as a primer for autologous red blood cell donation. This has increased the number of autologous units of blood donated and also produced a higher preoperative hematocrit reducing the need for RBC transfusion in orthopedic surgeries (Godnough *et al.*, 1989; Sparling *et al.*, 1996; Sharma *et al.*, 2013). In myelodysplastic syndromes inverse relationship between Epo levels and the degree of anaemia has been demonstrated in patients with endogenous Epo levels less than 100 U/1 and blast counts of less than 5% (Jacobs *et al.*, 1986; Greenberg *et al.*, 2009).

Short-lived improved response to r-HuEpo by patients with aplastic anaemia and high levels of endogenous Epo was observed when granulocyte colony-stimulating factor was incorporated during therapy (Hellstrom-lindberg *et al.*, 1997; Greenberg *et al.*, 2009). Erythropoiesis has been stimulated in patients with multiple organ failure, by administering r-HuEpo at 600 units/kg (Gabriel *et al.*, 1998; Sinclair, 2013). It has been found that anaemia attributed to protozoal disease is ameliorated by administration of Epo and also effective for treatment of protozoal disease itself. Therefore, drugs with Epo-containing pharmaceutical composition effective for the treatment of protozoal disease are being developed (Seiyaku and Hiroshi, 2005; Monzote and Siddiq, 2011). AIDS patients with endogenous Epo levels less than 500IU/L need fewer red cell transfusions, have higher HB level, and report an improvement in quality of life when treated with Epo

(Henry *et al.*, 2004; Sullivan, 2011). Some of adverse effects of Epo have been demonstrated in patients with chronic kidney disease and cancer and they include: increased thromboembolic complications, cardiovascular mortality and tumor progression (Unger *et al.*, 2010). Treatment of HIV-infected patients with Epo resulted in the development of haematologic toxicity where anaemia was due to chronic illness and opportunistic infections (Hoffbrand *et al.*, 2005; Redig and Berliner, 2013).

Recombinant Epo has been widely used in the management of anaemia in diseases like: chronic renal failure (Naicker, 2003; Legbo, 2016); cancer, myelodysplastic syndromes, bone marrow transplantation, hepatitis C (Winearls *et al.*, 1986; Eschbach *et al.*, 1987; Henry *et al.*, 2004; Delanghe *et al.*, 2008); multiple myeloma (Mittelman *et al.*, 1997; Rocchetta *et al.*, 2011); multiple organ failure (Mittelman *et al.*, 1997; Rocchetta *et al.*, 2011). Recombinant Epo has also been shown to decrease serum interleukin-6 levels (Prutchi-Sagir *et al.*, 2006). In addition, high levels of endogenous Epo with concurrent increases in TNF- $\alpha$  have been shown to be associated with anaemia (Hoffbrand *et al.*, 2005; Grossi *et al.*, 2007). Trends in serum levels of proinflammatory cytokines and endogenous Epo in different severities and types of HIV-associated anaemia have not been reported. This would have been pivotal in explaining the effect of HIV pathology on the normal Epo induced erythropoiesis.

### 2.11 Liver Function profiles in HIV infections

Increases in hepatic enzymes in HIV infected patients is possibly secondary to factors such as alcoholism, lipid lowering drugs, co-infection with hepatitis viruses, or hereditary diseases. Direct damage of hepatic cells by HIV has been reported (cappel, 1991; Marina *et al.*, 2001; Ogedegbe and Sulkowski, 2003; Castellares *et al.*, 2008). Measurement of hepatic enzymes is used to diagnose and assess liver activities. Alanine aminotransferase (ALT) catalyzes the transfer of amino groups to form hepatic metabolite oxaloacetate (Price and Albert, 1979; Wolf, 1999; Kim *et al.*, 2008). Measurement of its activity is a reliable and sensitive marker of liver disease. Most patients with abnormal ALT activity are asymptomatic resulting in the abnormality being ignored by many practitioners.

The main source of ALT is the cytosol of the hepatocyte. Other sources include kidney heart and skeletal muscle. Increased activity of ALT is seen following hepatocellular injury or death. The elevation of ALT activity persists longer than that of aspartate aminotransferase (AST). In health ALT levels in serum are: 7 - 45 U/L and 7 - 55 U/L in females and males respectively. The ration of ALT to AST is normally less than 1. The liver is also the main source of AST. Other sources include: heart, skeletal muscle and kidney. This enzyme is present in both cytoplasm and mitochondria of cells. Normally its levels in serum range from 8 - 48 U/L in males aged over 14 years and from 8 - 43 U/L in females of the same age gap. Increases in AST levels are found in myocardial infarction, acute liver cell damage, viral hepatitis and carbon tetrachloride poisoning. In alcoholism, there is coexistence of deficiency of pyridoxal-6-phosphate together with release of mitochondrial AST with longer half-life. This may explain why AST activity is characteristically elevated in comparison to ALT activity in alcoholic liver injury. (Diehl, 1984; Sorbi *et al.*, 1999; Thapa and Walia, 2007).

Gamma glutamyltransferase (GGT) is an enzyme found in the cell membranes of tissues such as kidneys, bile duct, pancreas, gallbladder, spleen, brain and seminal vesicles. This
enzyme plays a role in the transfer of amino acids across the cellular membrane and leukotriene metabolism. It also plays a role in extracellular catabolism of glutathione the main thiol intracellular antioxidant agent in mammalian cell (Dominic, 2005; Karakur, 2011). Measurement of GGT activity has been used to assess liver function and alcohol consumption. The enzyme is also considered as a marker of oxidative stress. Association of GGT with morbidity and mortality, including cardiovascular disease independent of liver disease or alcohol consumption has been reported (Whitefield, 2001; Pompella *et al.*, 2004; Ruttmann *et al.*, 2005; Jiang *et al.*, 2013).

Alkaline phosphatase is an enzyme that catalyzes the hydrolysis of phosphatase esters in an acidic environment and plays a role in transportation across cell membranes (Handan *et al.*, 2009). This enzyme is present in tissues such as intestine, kidney, liver, placenta and white blood cells. The reference levels of ALP in serum are: 44 to 147 U/L. High levels of ALP may be predictive of mortality independent of bone metabolism factors and liver functions in chronic kidney disease (Siriharsha *et al.*, 2011). There is association of elevated ALP levels with elevated CRP levels denoting that ALP can be a marker of inflammation. Elevated ALP levels are observed in: liver disease or bone disorder.

Bilirubin is formed from the breakdown of haem derived from senescent erythrocytes in mammals. It is a yellow compound and its reference ranges are: total bilirubin  $< 21 \mu mol/l$  (< 1.23 mg/dl) and direct bilirubin 1.0 -5  $\mu moles/l$  (0 – 0.3 mg/dl). It is excreted in bile and urine. Once conjugated with a molecule of glucuronic acid in the liver, it is termed "direct" bilirubin and is soluble in water. High levels of unconjugated bilirubin are seen in increased haemolysis. It has been shown that high levels of total bilirubin in absence of

liver disease is health wise beneficial (Sedlak *et al.*, 2009; Van Wagner and Green, 2015). Moreover, high levels of bilirubin in elderly have been associated with higher functional independence (Kao *et al.*, 2012). It has also been reported that levels of serum bilirubin are inversely related to risk of certain heart diseases (Navotiny and Vitek, 2003; McArdle *et al.*, 2012). Estimation of serum total protein is another test for liver function. In health serum protein levels range from 6.6 - 8.7 g/dl. Protein in plasma is made up of albumin and globulin with albumin comprising more than half of the fractions.

Albumin is distributed in the body in the intravascular compartment (approximately, 30 to 40%) and extravascularly in the interstitial spaces, in body fluids such as sweat, tears, gastric juice and bile. Albumin reference ranges are 3.8-5.1 g/dl. This protein fraction plays a major role in: regulating passage of water and diffusible solutes through the capillaries by providing colloidal or osmotic pressure, the transport of bilirubin, hormones, metals, vitamin and drugs, and in lipid metabolism by binding fatty acids and keeping them in soluble form in the plasma. Albumin is synthesized in the liver. The only clinical situation that cause elevated serum albumin levels is acute dehydration and decreases are due to depressed synthesis as seen in end - stage liver disease or increased loses as seen in nephritic syndrome. Proteins that comprise the globulin fraction include: enzymes, complement and immunoglobulins. Except immunoglobulins, these proteins are synthesized in the liver. Decrease in protein is found in malnutrition and congenital immune deficiency due to decreased synthesis, and in nephritic syndrome due to loss through the kidney (Guillen et al., 1996; Braamskamp et al., 2010). Liver toxicity has been reported to be associated with excess lipid deposition on the membrane of erythrocytes resulting in the presence of round macrocytes in peripheral blood (Veda, 2012). The state of liver functions in the course of HIV- associated anaemia need to be monitored to aide in understanding the etiology of the morphological types of anaemia in HIV.

#### 2.12 Kidney Function profiles in HIV-infections

Renal disease has been reported as an important complication in HIV with up to 30% of the patients demonstrating abnormal kidney function. End –stage renal disease in HIV has become relatively common and is associated with progression to AIDS and death (Agbaji et al., 2011; Scarpino et al., 2015). Health care providers need to identify HIV infected patients at risk of renal disease in order to implement potentially preventative and therapeutic strategies. This makes regular assessment of kidney functional status in HIV an essential component of the disease management. The main by products that are assayed to assess kidney function are urea and creatinine. Creatinine is a byproduct of meat protein metabolism. Meat protein can be derived from the diet and from normal wear and tear on muscles of the body. The normal ranges of creatinine are  $53 - 97 \mu$ moles/l and 44 - 80 µmoles/l in males and females respectively. Increased levels occur in kidney disorders. Conversely, the breakdown of food protein yields urea. Reference ranges of blood urea are 1.7 - 8.3 mmoles /l. Increased levels are indicative of decreased kidney function. The kidney is the main source of endogenous erythropoietin (Haase, 2010; Debevec et al., 2012), the obligatory factor in erythroid development (Amanzada et al., 2014; Beverborg et al., 2015). Monitoring the state of kidney function and the trend in serum levels of endogenous erythropoietin in HIV associated anaemia would provide information for differentiating etiologies of the anaemia between reduced Epo production and Eporestricted erythropoiesis.

#### 2.13 Reference Intervals for Clinical values

Reference intervals are defined as the variations of measurements in normal healthy individuals (Ichihara et al., 2013; Smith et al., 2019). Their role in research and diagnostic services cannot be under scored. For proper interpretation of laboratory results and accurate clinical decisions, appropriated reference intervals are absolutely necessary. Numerous reports indicate that due to variations in: race, geographical locations, sex, altitude, climate, diet, and environment; reference intervals significantly vary between African populations and between Africans and Caucasians (Ngovi et al., 2009; Mine et al., 2011; Addai-Mensah et al., 2019). For example, a study conducted among four ethnic groups in United Kingdom demonstrated that black women had significantly lower white cell and neutrophil counts compared to Indian, Northern European and Oriental women (Bain et al., 1984; Kibaya et al., 2008). In addition, a study carried out at Kericho, Kenya, which is approximately 2042 m above sea level showed that the participants had lower haemoglobin ranges compared with Tanzanians and Ethiopians; higher ALT and AST ranges compared to Tanzanians and North Americans (Kibaya et al., 2008). Waithaka et al. (2009) demonstrated that Kenyan blood donors in Nairobi have higher ranges for: protein, albumin, phosphorus potassium and sodium compared to Kuwaitis and Americans. In these studies significant gender-based variations in clinical values were generally demonstrated.

Africans have lower, haemoglobin, red blood cell counts, haematocrit, mean cell volume, platelets and neutrophils but higher monocytes and eosinophil values than Caucasians (Urquhart *et al.*, 2008; Lawrie *et al.*, 2009; Yalew *et al.*, 2016).

To minimize the risks of influencing the results obtained in this study by the unique peculiarities of individuals, reference intervals in the study were derived from HIV negative blood donors with similar social demographic characteristics as the HIV infected patients

#### **CHAPTER THREE**

#### MATERALS AND METHODS

#### 3.1 Study site

This study was carried out at Kenyatta National Hospital (KNH) Comprehensive Care Center (CCC) between 2013 and 2016. The hospital was started in 1901, and is the largest hospital in Kenya and East Africa. The institution is a teaching and referral hospital, providing specialized medical care, training and research. The hospital is a public facility situated in Nairobi County, and offers services that are subsidized by the government. Moreover, Nairobi County has HIV prevalence rates that are comparable to the national prevalence at 6.1% (National AIDS Control Council, 2016). This makes the hospital the most likely health facility to capture disease patterns suitable for making population-based inferences for Kenya. Hence the choice of the site for the study.

#### **3.2 Experimental design**

This was a prospective cohort study on HIV positive respondents and a cross-sectional study on HIV-seronegative respondents.

#### 3.3 Study population

The study was carried out on HIV positive respondents attending comprehensive care clinic (CCC) at Kenyatta National Hospital and HIV-negative blood donors from the blood donor unit of the hospital. The cases had been screened through the routine laboratory protocols of the hospital for HIV and their confirmed seropositive status documented before enrollment for the clinic. Social demographic characteristics on smoking, alcohol – drinking, occupation status and the level of education were noted. The tools to capture

patient's information included: patients file records and pre-designed structured questionnaires.

# 3.4 Inclusion and exclusion criteria for HIV positive respondents

# **3.4.1 Inclusion criteria**

- i. HIV-seropositive adults aged between 18 and 60 years who gave informed consent.
- ii. Both ARV-treated and ARV-naive patients with no symptomatic renal or liver disorders.

# **3.4.2 Exclusion criteria**

- .i. Non- consenting patient.
- .ii. HIV-positive patient above 60 years or below 18 years.
- iii. Patients who had been transfusion with blood within the last one month.
- iv. Pregnant women.
- v. Breast feeding mothers

# 3.5 Inclusion and exclusion criteria for HIV negative respondents

# **3.5.1 Inclusion criteria**

- i. Consenting adults aged between 18 and 60 years.
- ii. Adults with normal renal and liver functions.

# 3.5.2 Exclusion criteria

- i. Individuals who had been transfused with blood within the last one month.
- ii. Pregnant women.

#### iii) Breast feeding mothers

#### **3.6 Sample size calculation**

The sample size was calculated using the following formula by Fisher et al. 1991:

$$=\frac{Z^2pq}{d^2}$$

Where:

n	=	Minimum sample size
р	=	Prevalence of the condition
q	=	1 - p
Ζ	=	Value corresponding to 95% confidence interval which equals
		1.96

d = Level of precision set at 
$$0.05 (5\%)$$

Kenya is a developing country and was assumed to have HIV associated anaemia at prevalence rate of between 80 - 90% (Semba and Gray, 2001; Kasthuri *et al.*, 2006; Yesuf *et al.*, 2019).

At 80 %: 
$$\frac{1.96 \ x \ 1.96 \ x \ 0.8 \ x \ (1.0 - 0.8) = \ 245.9}{0.05 \ x \ 0.05}$$

At 90 %: 
$$\frac{1.96 \times 1.96 \times 0.9 (1.0 - 0.9)}{0.05 \times 0.05} = 138.3$$

A sample size of between 138 and 246 would be acceptable for the study. In this case a sample size of 184 (119 females and 65 males) was selected and recruited consecutively. Stratification of sample size by sex and CD4+ counts was done after recruitment and base line CD4 determination respectively. The respondents were then categorized into three

groups based on Centers for Disease Control (CDC) baseline CD4+ cells / mm<sup>3</sup> - definition of AIDS: CD4+ cell counts of < 200 cells / mm<sup>3</sup> (15 females and 6 males), 200 - 499 cells / mm<sup>3</sup> (54 females and 33 males) and CD4+ cell counts of  $\geq$  500 cells / mm<sup>3</sup> (50 females and 26 males) (Castrol *et al.*, 1993). The groups were followed after three then after six months and denoted as: baseline (F<sub>0</sub>), 3 months (F<sub>1</sub>) and 6 months (F<sub>2</sub>). Haematological, biochemistry, Epo, TNF- $\alpha$ , IL-6 and CRP parameters for each visit were analyzed. Blood samples were identified by study numbers only.

A total of 243 HIV negative blood donors (referents) comprising of 122 females (50.2%) and 121 males (49.2%) were recruited according to Clinical and Laboratory Standards Institute recommendations of the minimum sample size of 120 for reference intervals determination (CLSI) 2008). The parameters of the referents were used as reference ranges in the study.

#### **3.7 Ethical considerations**

- Permission to undertake the study was obtained from the Ethics and Research Committees of KNH-UoN (Appendix 1).
- iii. Informed consent was obtained from the study participants and copies of the signed consent forms given to them (Appendix 2).
- iv. Confidentiality was maintained at all stages of the study by using study numbers for identification.
- v. Extensions of the study periods were permitted by KNH-UoN Research and Ethics Committees.

#### **3.8 Blood collection**

After collection of demographic and clinical information, a 5 ml sample of blood was collected aseptically from the cubital vein using a sterile gauze 21 needle then divided equally into ethylene diamine tetra acetic acid (EDTA)- containing tubes and into plain sample tubes and assigned study codes. The code was entered into a list, a copy of which was kept by the investigator. In the subsequent clinic visits, suffix letters were added to the study code to identify the visit; suffix "A" for the first visit and "B" the second. From the EDTA sample, CD4 +cell counts and blood counts were determined. Plasma from the remaining samples was separated and used for Epo, IL-6 and TNF- $\alpha$  assays. Serum was obtained from samples in the plain tubes by spinning at 760 x g for 15 minutes and stored in aliquots at  $\leq -20^{0}$ C for CRP, Urea, creatinine (CRT) and liver function (LF) assays. Referent samples were treated similarly.

#### **3.9 Experimental procedures**

#### 3.9.1 Erythropoietin assay using a double-antibody sandwich ELISA

In this assay anti-human Epo precoated wells were incubated with 100µl of plasma or standard for two and half hours at 18 - 25 <sup>0</sup> C (room temperature) and then the contents aspirated out. The wells were washed four times using buffer and an automatic washer. After drying the wells 100µl of biotinylated antibody reagent was added to each well and incubated for 1 hour at 18 - 25 <sup>0</sup> C with gentle shaking. The wells were then emptied and the washing repeated. Into the wells 100µl of streptavidin - horseradish peroxidase was added and then incubated at 18 - 25 <sup>0</sup> C for 45 minutes. The contents were discarded and washing repeated. After drying the wells, 100µl of substrate reagent (TMB substrate) was added to each well and after 30 minutes of incubation at 18 - 25 <sup>0</sup> C, the reaction was

stopped using 0.2 M sulphuric acid. The absorbance was measured on an ELISA reader set at 450 nm. The concentration of erythropoietin in pg./ml was read off the standard curve. The standard curve was constructed by determining the absorbance of serially diluted commercial standard and plotting the absorbance on the Y- axis and the concentrations on the X-axis. Quality control was maintained using the commercial standards.

#### **3.9.2 CD4+ cell counts analysis**

This analysis involved addition of a 50µl aliquot of whole blood to fluorochrome- labeled antibodies and allowed to stain for 15 minutes in the dark at  $26^{\circ}$  C. A 50µl volume of fixative was then added and fixation allowed for 30 minutes, then fed into a FACS CALIBUR® machine. The instruments utilize the information generated by the fluoresce of fluorochrome labeled cells as they come into contact with the laser light to count the cells. The software identifies T lymphocyte populations and calculates the CD4+ cells, displays and prints them automatically as numbers per microliter (Giorgi and Hultin, 1990). The reagent tubes also contain known number of fluorochrome integrated reference beads for locating the lymphocytes and quantifying them. Commercial controls set at zero, low, medium and high concentrations maintain the quality control of the instrument.

#### 3.9.3 Haematological analysis

Approximately  $30\mu$ l aliquot of EDTA-anticoagulated blood was aspirated by a Cell TAC F Model 822® analyzer and the white blood cells (WBCs) and platelets automatically counted and expressed as number of cells x 10 <sup>9</sup>/l; while red blood cells (RBCs) were

expressed as number of cells x 10<sup>12</sup>/l. Haemoglobin was expressed in grams per deciliter (g/dl); mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) in g/l and mean cell volume (MCV) as femtoliters (Fl). Quality control was maintained through daily running of tri level (normal, low and high) commercial controls. Levy Jennings curves were plotted bimonthly to monitor the precision of the instrument and standard deviation (SD) values within ±3 and coefficient of variation (CV) of  $\leq$  30 % taken as acceptable.

#### **3.9.4 Peripheral blood film examination**

Leishman's stain was the Romanowsky stain that was used to stain blood films. Dried blood films were fixed for 3 minutes with neat stain, and then stained for 10 minutes with diluted stain at  $P^{H}$  6.8 buffer. Films were rinsed in water, air-dried then examined microscopically.

#### 3.9.5 C-Reactive Protein immunoturbidimetric test

The plasma sample was aspirated into a fully automated instrument, Humastar 600® (Appendix 6), and analysis done automatically. The instrument was calibrated using 2 levels of "Turbidos" control and commercial standards were used to ensure reproducibility of the result. Levy-Jennings curves plotted using cumulative readings were used to monitor the validity of the results with standard deviation of  $\pm$  3 and coefficient of variation of  $\leq$  30 % taken as the cut-off values.

#### **3.9.6.** Test for creatinine

Serum samples or standard were arranged in racks and a mixture of diluted sodium hydroxide and picric acid placed in reagent bottles placed in reagent positions in the analyzer. The analyzer did the mixings automatically. The optical density of the reaction was read at 492 nm (490-510) in a fully automated HUMASTAR: 600 ® analyzer. The readings displayed and printed. The results were expressed as micromoles per liter (µmoles/l). Normal and pathological controls were used for quality control.

#### 3.9.7 Test for blood Urea

Serum samples and standards were placed in racks and positioned in a fully automated HUMASTAR: 600 B, analyzer. The reagents comprising of enzyme urease, salicylate in phosphate buffer (PH 7.0) and hypochlorite in phosphate buffer (PH < 13) were placed in the instrument and analyzes done automatically., results displayed on the monitor' screen and then printed. The results were expressed as millimoles per liter (mmoles/l). Commercial normal and pathological controls were used for quality control.

#### 3.9.8 Interleukin- 6 assay

In this assay a 50 $\mu$ l aliquot of biotinylated antibody reagent was added into each of the wells precoated with antihuman II-6 antibody. Then 50 $\mu$ l aliquot of reconstituted standard or specimen were added and mixed by gently tapping the plate several times. The plates were carefully covered by adhesive plate covers and incubated for 2 hours at 20-25 <sup>0</sup> C. The adhesive plate covers were carefully removed and the plates washed 3 times with wash buffer on automated washer shown in appendix 5. Aliquots of 100  $\mu$ l of freshly reconstituted Streptavidin-Horse Radish peroxidase (HRP) solution were added into each well and a new adhesive plate cover attached carefully ensuring that all edges and strips are tightly sealed then incubated at 20-25° C for 30 minutes. The adhesive plate covers were carefully removed, and the plates washed 3 times as in the previous washing steps. Aliquots 100  $\mu$ l of chromogen substrate (TMB substrate) were added into each well and color reaction allowed to develop at room temperature in the dark for 30 minutes. The

reaction was stopped by adding 100  $\mu$ l of 0.16 M sulfuric acid. The absorbance of the final yellow color was read at 450 nm on a HumaReader HS Elisa reader and concentration of interleukin- 6 in pictograms per milliliter (pg./ml) read off the standard curve. The standard curve was constructed by determining absorbance of serially diluted standard and plotting of absorbance values against the standard concentration values in pg. /ml.

#### 3.9. 9 Tumor necrosis factor-alpha assay

Tumor necrosis factor-alpha was determined using ThermoScientific® quantitative sandwich enzyme immunoassay technique. A 50µl aliquot of sample diluent was added to each well, pre-coated with anti-human TNF-α. Then a 50µl of standard or specimen was also added to the wells, mixed well, tightly covered with a plate adhesive cover and incubated at 20-25<sup>0</sup> C for 1 hour. The adhesive cover was carefully removed and the wells washed 3 times with wash buffer and an automated washer. Aliquots of 100 µl biotinylated antibody reagent were added to each well. The plates were tightly covered with an adhesive plate cover and the preparation incubated for 1 hour at  $20 - 25^{\circ}$  C. The adhesive cover was then removed and the wells washed as previous. Aliquots of 100 µl Streptavidin - HRP reagent were added into each of the wells, the plates tightly covered with adhesive covers and incubated at  $20 - 25^{\circ}$  C for 30 minutes. The adhesive cover was carefully removed, washing done as previous. Aliquots of 100 µl TMB substrate solution were added into each of the wells and the enzymatic reaction to develop color allowed at 20 -25<sup>°</sup> C for 30 minutes. The reaction was stopped with 100 µl 0.16 M sulfuric acid and absorbance was read within 30 minutes using at 450 nm on HumaReader HS® Elisa reader. Concentration of tumor necrosis factor – alpha in pg./ml was read off the curve constructed using serially diluted TNF-  $\alpha$  commercial standard.

#### **3.9.10** Assay for direct bilirubin

Serum samples and standards were placed in racks and positioned in a fully automated HUMASTAR: 600 <sup>®</sup> analyzer. Reagents comprising of a mixture of: sulphuric and hydrochloric acid, nitrite reagent were placed in reagent baths in the analyzer. Mixing of the samples and the reagents, incubation and reading of the absorbance were done by the analyzer automatically. After the display of the results, the instrument printed the results expressed in µmoles/l. Normal and pathological controls were done for quality control.

#### **3.9.11** Assay for total bilirubin

Serum samples and standards were placed in racks and positioned in a fully automated HUMASTAR: 600 <sup>®</sup> analyzer. In addition, reagents comprising of sulphuric acid, hydrochloric acid, caffeine, sodium azide sodium nitrite were positioned in the analyzer. The analyzer mixed the samples and the reagents, incubated, read the absorbance at 546 nm, displayed the results in and printed them. Absorbance was read automatically at 546 nm and the results expressed in. µmoles/l. Normal and pathological controls were used for quality control.

#### **3.9.12** Assay for L-gamma (γ)-glutamyl transferase (GT)

Serum samples and standards were placed in a fully automated HUMASTAR: 600 @ analyzer. Reagents comprising of L- $\gamma$ glutamyl-3-carboxy-4-nitroanilide and glycylglycine in Tris buffer (PH 8.3) were also positioned in the analyzer. The analyzer mixed samples and reagents, incubated and read the absorbance at 405 nm, displayed the results in units per liter (u/l) and printed them. Commercial normal and pathological controls were used for quality control.

3.9.13 Assay for alanine aminotransferase /Glutamyl pyruvate transferase (GPT)

Serum samples and standards were placed in racks and positioned in a fully automated HUMASTAR: 600 <sup>®</sup> analyzer. Reagents comprising of Tris buffer (PH 7.4), enzyme reagent and substrate were also positioned in the analyzer. The analyzer mixed the sample and the reagents, incubated and read the absorbance at 365 nm automatically. The results were displayed as u/l and printed. Commercial normal and pathological controls were used for quality control.

#### 3.9.14 Assay for aspartate aminotransferase/Glutamate oxaloacetate transferase

Serum samples and standard were placed in fully automated HUMASTAR: 600 ® analyzer. Reagents comprising enzyme reagent in Tris buffer (PH 7.9), enzyme substrate, (2-oxoglutarate and NADH) were also positioned in the analyzer. The analyzer mixed the samples and the reagents, incubated, read the absorbance read at 365 nm, displayed the results in u/l and printed them automatically. Commercial normal and pathological controls were used for quality control.

#### **3.9.15 Determination of total protein**

Serum samples and standards were placed in a fully automated HUMASTAR: 600 ® analyzer. Color reagent comprising of sodium hydroxide, potassium sodium tartrate, copper sulphate and potassium iodide was also placed in the analyzer. Mixing of the samples and the reagents, incubation, reading of the absorbance, display of the results in g/l and printing them was done automatically by the analyzer. Commercial controls were used to maintain quality control.

#### **3.9.16Albumin** assay

Serum samples and standards were placed in Humastar 600 ® analyzer. Citrate buffer (PH 4.2) and bromocresol green reagent were also positioned in the analyzer. The analyzer mixed the sample and the reagents, incubated, read the absorbance at 546 nm, displayed the results in g/l and printed them automatically.

#### 3.9.17Alkaline phosphatase (AP) test

Serum samples and standards were placed in fully automated HUMASTAR 600  $\circledast$ . P-Nitrophenyl phosphate and magnesium chloride in diethanolamine buffer (PH 3.5± 0.2) were also placed in the analyzer. The analyzer mixed the samples and the reagents, incubated, read the absorbance at 405 nm, displayed the results in u/l and printed them automatically.

#### 3.10 Quality assurance

Analyses were carried out in a laboratory that participates in an external quality control scheme based in United Kingdom. Internally, trilevel controls (low, normal and high) monitored the precision of the haematological analyses. Quality control for Fluorescence Activated Cell Sorting (FACS) CALIBOUR analyzer for CD4+ counts determinations was monitored using normal and abnormal commercial controls and Levy Jennings graphs constructed with a maximum of 3 –standard deviations from the control means considered as acceptable (appendix 7(i)). Biochemistry and haematological assays were run after commercial controls met the manufacturer's recommended ranges (Appendices 4 and 5). C-reactive protein assays were controlled by Turbidos ® commercial controls. Erythropoietin, interleukin-6 and tumor necrosis factor-alpha were determined from

standard curves constructed using commercial standards. The standard readings were carried out in duplicates. The analyses were carried out by qualified laboratory personnel.

#### 3.11 Data management and statistical analysis

The raw data was transferred from a laboratory note book into excel computer data base for cleaning and verification. Then the data was transported into statistical package for the social sciences (SPSS) version 21 for analysis The fit of the observed distribution was determined using Shapiro – Wilk tests with P > 0.05 (Gaussian distribution) being considered significant. Bootstrap parametric and non-parametric methods were used to calculate means and medians of referents to raise the power of the low sample sizes. Medians, were used to describe non-parametric parameters, while means and standard deviations were used to describe data with Gaussian distribution. Frequency was used to describe the number of values above and below  $2.5^{\text{th}} - 97.5^{\text{th}}$  percentile ranges as percent increases and decreases respectively. Wilcoxon rank-sum test was used to compare values between referents (F<sub>N</sub>) and baseline (F<sub>0</sub>) values and values at different follow-up stages in the same CD4 groups. Kruskal-walis test was used to compare parameters in the same follow-up stage in the different CD4 groups (between CD4 groups' comparisons). Blood cell Morphological findings were presented in photomicrographs and percent frequencies

#### **3.11 Study Limitations**

Randomization was not done during recruitment. This may have reduced the strength of making population-based inferences on the studied characteristics. Details of ARVs and other drug regimens for HIV co-infections used by the HIV positive respondents were not taken into account. The effects of the drugs on the study outcomes were thus not well elucidated. This would however, be best dealt with in clinical trials- based studies. Study

participants were not stratified by ages. Studies involving Epo and proinflammaory cytokines among Africans are few and this hindered inter-laboratory comparison of the results obtained.

#### **CHAPTER FOUR**

#### RESULTS

#### **4.1 Development of reference ranges for the HIV negative control population**

#### **4.1.1** Social demographics of the HIV negative control population

A total of 243 HIV seronegative blood donors comprising of 121 (49.8%) males and 122 (50.2%) females were recruited as referents for this study. The sample size was in conformity with the Clinical Laboratory Standards Institute that recommends a minimum sample of 120 for development of reference ranges of a population (CLSI) 2008). The mean age for the group was  $31.84 \pm 9.0$  and ranged from 18 to 60 years.

The majority of the participants had tertiary education (50.6%); while the rest had: secondary (36.2%), primary (12.3%) or no academic qualification at all (0.8%). Of the participants 37.9% were businessmen, 29.6% were in formal employment, 25.9% were students, 4.9% were house wives and the rest had no defined occupation. A total of 97.5% of the participants were non- smokers and 83.1% were non-alcohol drinkers (Table 4.1).

HIV – negative (N=243)										
Variable		Mean ± SD	Min- Max	Frequency (%) (N = 243)						
Age (Yrs.)		$31.84\pm9.0$	18 - 60							
Education										
	None			2 (0.8%						
	Primary			30 (12.3%						
	Secondary			88 (36.2%						
	Tertiary			123 (50.6%)						
Occupational										
	None			4 (1.6%						
	House			12 (4 9%)						
	wife			12 (11970)						
	Business			92 (37.9%)						
	Employed			72 (29.6%)						
	Student			63 (25.9%)						
Smoking										
	Yes			6 (2.5%)						
	No			237 (97.5 %)						
Alcohol										
	Yes			41 (16.9%)						
	No			202 (83.1%)						
ARV-treated										
	Yes			-						
	No			-						

 Table 4.1: Social demographic characteristics of the referents

#### **4.1.2 Reference ranges of blood parameters of the control population**

During analysis, data for 14 (11.5%) females and 37 (30.6%) males was excluded because the respondents were found to be smokers, alcoholics or both. Therefore 108 females and 94 males were considered in the determination of the reference ranges. These numbers were below the sample size of 120 recommended for establishing reference ranges (CLSI, 2008). Nonetheless this was compensated for by using parametric and non-parametric bootstrap methods for parametrically and non-parametrically distributed data respectively. (Coskun *et al.*, 2013).

# 4.1.2.1 Immonuhaematological parameters

Males demonstrated significantly higher mean values than females in: RBCs ( $5.07 \times 10^{12}/1$  versus  $4.42 \times 10^{12}/1$ , p< 0.001), HB (14.89 g/dl versus 12.92 g/dl, p< 0.001), and E ( $0.3 \times 10^{9}/1$  versus  $0.1 \times 10^{9}/1$ , p< 0.001). Significantly lower mean values in males than in females were, however, demonstrated in: WBC ( $4.99 \times 10^{9}/1$  versus  $5.59 \times 10^{9}/1$ , p = 0.002), N ( $2.1 \times 10^{9}/1$  versus  $2.5 \times 10^{9}/1$ , p = 0.004), L ( $2.2 \times 10^{9}/1$  versus  $2.5 \times 10^{9}/1$ , p = 0.001), M ( $0.4 \times 10^{9}/1$  versus  $0.5 \times 10^{9}/1$ , p = 0.003), CD4+ cells ( $702/\mu$ l versus  $848/\mu$ l, p< 0.001) and P ( $234 \times 10^{9}/1$  versus  $256 \times 10^{9}/1$ , p< 0.001) (Table 4.2 and Figures 4.1- 4.5)..

Parameter	Sex	Ν	Mean	SD	95% CI	p-value	Mode	Median	2.5 <sup>th</sup> -97.5 <sup>th</sup>
	Combined	202	4.72	0.69	46 - 4.8		4.9	4.7	3.4 - 6.14
RBCx1012/1	Female	108	4.42	0.56	4.3 - 4.5	< 0.001	4.3	4.42	3.4 - 5.8
	Male	94	5.07	0.65	4.95.3	< 0.001	5.2	5.11	3.0 - 6.22
	Combined	202	13.83	2.09	13.5 - 15		14.2	13.8	9.53-17.99
HB g/dl	Female	108	12.92	1.76	12.6 - 13.3	- 0.001	13.8	12.85	9.3-17.6
	Male	94	14.89	1.94	14.5 - 15.3	< 0.001	15.1	15.1	9.5 - 18.2
	Combined	202	88.04	6.23	87.2 - 88.9		88.5	89	72.91 - 97.99
MCV fl	Female	108	88.0	6.9	86.5 - 89.3	0.092	88.5	88.7	71.1 - 99.5
	Male	94	88.0	5.5	86.9 - 89.2	0.985	91.1	89.2	75.1 - 98.0
	Combined	202	29.33	2.67	28.96 - 29.7		30.1	29.7	23.4-34.3
MCH g/l	Female	108	29.3	2.8	28.8 - 29.3	0.702	30.1	29.9	23.7 - 34.3
-	Male	94	29.4	2.6	28.9 - 30.0	0.795	30.1	29.7	22.8 - 34.3
	Combined	202	33.43	2.09	33.1 - 33.7		34.1	33.4	30.2 - 35.9
MCHC g/l	Female	108	33.5	2.4	33.0 - 34.0	0.602	33.5	33.4	30.8 - 38.2
0	Male	94.0	33.4	1.7	33.0 - 34.0	0.623	34.1	33.5	29.2 - 41.9
	Combined	202	5.31	1.36	5.12 - 5.50		5.6	5.16	2.8 - 8.5
WBC x109/l	Female	108	5.59	1.35	5.3 - 5.8	0.002	5.6	5.3	3.4 - 3.8
	Male	94.0	4.99	1.3	4.7 - 5.3	0.002	5.5	5.0	2.3 - 7.8
	Combined	202	2.35	0.96	2.2 - 25		1.8	2.1	0.91-4.7
N x10 <sup>9</sup> /l	Female	108	2.5	1.0	2.3 - 2.7	0.004	1.8	2.4	1.1 - 5.1
	Male	94	2.1	0.9	2.0 - 2.3	0.004	2.0	2.0	0.7 - 5.3
	Combined	202	2.35	0.64	2.26 - 2.44		2.7	2.4	1.1 - 3.4
L x10 <sup>9</sup> /l	Female	108	2.5	0.6	2.4 - 2.6	0.001	2.7	2.5	1.6 - 3.4
	Male	94.0	2.2	0.7	2.0 - 2.3	0.001	2.0	2.2	1.0 - 4.1
	Combined	202	0.47	0.3	0.43 - 0.51		0.3	0.4	0.i - 1.3
M x10 <sup>9</sup> /l	Female	108	0.5	0.3	0.45 - 0.56	0.002	0.3	0.4	0.2 - 1.5
	Male	94	0.4	0.3	0.37 - 0.47	0.003	0.4	0.4	0 - 1.3
	Combined	202	0.13	0.24	0.10 - 0.16		0.0	0.0.	0 - 0.89
E x10 <sup>9</sup> /l	Female	108	0.1	0.1	0.04 - 0.09	.0.001	0.0	0.0	0 - 0.6
	Male	94	0.2	0.3	0.14 - 0.26	< 0.001	0.0	0.1	0 - 1.3
	Combined	202	779.76	295.48	738.8 - 820.8		569	758.5	282.3 -1442.3
CD4+ cells/µl	Female	108	848	289	793 - 903	0.001	791	839	216 - 1541
•	Male	94	702	284	643 - 760	< 0.001	542	667	278 - 1697
	Combined	202	245.6	77.3	234.9 - 256.3		200	244	111.2 - 425.3
P x 10 <sup>9</sup> /l	Female	108	256	73	242 - 270	0.001	182	253	114 - 446
	Male	94	234	80	217 - 250	< 0.001	184	239	92 - 473

Table 4.2: Means, standard deviations, medians, modes, 95% CI, 2.5 th -97.2 th percentiles and p-values of female and

RBC= Red blood cells, HB= heamoglobin, MCV=Mean cell volume, MCH= mean cell haemoglobin, MCHC = Mean cell haemoglobin concentration, WBC= White blood cell/ N = Neutrophil, L = lymphocyte, M= Monocyte, E = Eosinophil, CD4= Complementarity determinant 4 and P = Platelet Independent t-test p < 0.05 considered significant (bold).

male



Figure 4.1: Differences between female and male red blood cell mean values



Figure 4.2: Differences between female and male haemoglobin mean values



Figure 4.3: Differences between female and male WBC, N, L M and E mean values



Figure 4.4: Differences between female and male CD4+ cells mean values



Figure 4.5: Differences between female and male platelet mean values

#### 4.1.2.2 Biochemical parameters

Significantly higher Biochemical mean values in male than in females were demonstrated in: AST (28 u/l versus 24 u/l, p = 0.001), ALT (21 u/l versus 15 u/l, p < 0.001), ALP (101.9 u/l versus 83.5 u/l, p< 0.001), T.Bil (7.94 µmoles /l versus 6.43 µmoles /l, p = 0.004), D.Bil. (3.9 µmoles /l versus 2.73 µmoles /l, p< 0.001), ALB (46 g/l versus 39.6 g/l, p< 0.001) and CRT (99.2 mmoles //l versus 88.2 mmoles //l, p = 0.001). Conversely significantly lower mean values in males than in females were demonstrated in: T.PRT (69 g/l versus 79 g/l, p< 0.001) (Table 4.3 and Figures 4.6 – 4.9)

Parameter	Sex	Ν	Mean	SD	95 % CI	p-value	Mode	Median	2.5 <sup>th</sup> -97.5 <sup>th</sup>
	Combined	202	26.13	8.47	24.95 - 27.30		22	25	14.0 -46.62
AST u/l	Female	108	24.00	7.00	23.1 - 25.6	0.001	22	24	14.0 - 42.0
	Male	94	28.00	10.00.	26.1 - 30,.2	0.001	24	26	16 .0- 64.0
	Combined	202	17.87	11.28	16.31 - 19.44		13	15.5	5.0 - 48.9
ALT u/l	Female	108	15.00	8.00	13.3 - 16.3	< 0.001	13	13	4.0 - 38
	Male	94	21.00	13.00	18.5 - 24.1	< 0.001	17	18	7.0 - 70.0
	Combined	202	26.83	21.88	23.8 - 29.9		15	21	7.0 - 92.6
G-GT u/l	Female	108	26.60	20.50	22.7 - 30.6	0.002	15	22.5	7.0 - 102.0
	Male	94	27.00	23.50	22.2 - 31.8	0.902	17	20	7.0 - 138.0
	Combined	202	92.10	35.89	87.0 - 97.0		83	88	30 - 171.3
ALP u/l	Female	108	83.50	34.50	77.0 - 90.0	< 0.001	53	84	21.0 - 172.0
	Male	94	101.90	35.10	95.0 - 101.0	< 0.001	83	99	31.0 - 187.0
T DIL umalas	Combined	202	7.13	3.77	6.61 - 76.5		4.57	6.25	2.6 - 18.7
	Female	108	6.43	3.19	5.8 - 7.0	0.004	4.51	5.49	2.15 - 14.56
/1	Male	94	7.94	4.23	7.1 - 8.8	0.004	8.13	7.01	2.86 - 22.05
	Combined	202	3.14	1.51	2.93 - 3.34		1.71	2.81	1.71 -6.88
D.Bil µmoles /l	Female	108	2.73	1.04	2.5 - 2.9	< 0.001	1.71	2.5	1.71 - 5.47
	Male	94	3.60	1.80	3.2 - 4.0	< 0.001	1.71	3.42	1.71 - 11.58
	Combined	202	73.54	10.205	72.1 - 75.0		71	72	57.0 - 96.0
T.PRT g/l	Female	108	77.00	11.00	74.9 - 79.2	< 0.001	71	77	58.0 - 104.0
	Male	94	69.00	7.00	68.1 - 70.9	< 0.001	68	69	57.0 - 87.0
	Combined	202	43.94	4.98	43.2 - 44.6		41	44	36.0 - 53.0
ALB g/l	Female	108	42.00	5.00	41.5 - 43.4	< 0.001	41	41	34.0 - 53.0
	Male	94	46.00	4.00	47.7 - 46.6	< 0.001	44	45	38.0 - 55.0
	Combined	202	39.60	1.77	3.71 - 4.20		4	3.5	2.1 - 9.15
UREA µmoles /l	Female	108	3.80	1.50	3.5 - 4.1	0.122	3.1	3.4	2.1 - 8.4
	Male	94	4.20	2.00	3.7 - 4.6	0.155	4	3.8	2.2 - 10.8
	Combined	202	93.33	22.92	90.1 - 96.5		86	90	52.2 - 146.9
CRT mmoles //l	Female	108	88.20	23.20	83.8 - 92.6	0.001	86	86	50 - 173
	Male	94	99.20	21.20	94.9 - 103.6	0.001	88	97	70.0 - 159.0

Table 4.3: Means, standard deviations, medians, modes, 95% CI, 2.5 th -97.2 th percentiles and p-values of female and male Biochemical parameters

Comparison of male and female parameters using Independent t-test p < 0.05 considered significant. Bold denotes significant AST= Aspartate transaminase, ALT = Alanine aminotransaminase, GGT= gamma-glutamyl transaminase, ALP= alkaline phosphatase T.Bil= Total bilirubin, D.Bil = Direct Bilirubin, T.PRT= Total protein, ALB. = Albumin, CRT= Creatinine



Independent t-test, p < 0.05 considered significant

Figure 4.6: Differences between female and male AST, AIT and ALP mean values





Figure 4.7: Differences between Female and male T.Bil and D.Bil mean values



Independent t-test, p < 0.05 considered significant

Figure 4.8: Differences between female and male T.PRT and ALB mean values



Independent t-test, p < 0.05 considered significant

Figure 4.9: Differences between female and male creatinine mean values

# 4.1.3 Reference ranges of erythropoietin, C-reactive protein, tumor necrosis Factor-alpha and interleukin-6 in the control population

Significant increases in cytokine median values in male referents above the females were demonstrated in: Epo (4.39 u/ml versus 0.45 u/ml, p< 0.001), IL-6 (0.95 pg/ml versus 0 pg/ml, p = 0.016) and TNF –  $\alpha$  (4.55 pg/ml versus 0.0 pg/ml, p< 0.001): while significant decreases below the females were demonstrated in CRP (4.40 mg/ml versus 11.70 mg/ml, p< 0.001) (Table 4.4)

Donomotor	Sor	NT	Maan	6D	Mada	Madian	Ζ-	Р-	Dongo	2.5th 07.5th
Parameter	Sex	IN	Mean	<b>SD</b>	Mode	Meulan	value	value	Kange	2.5 - 97.5 -
	Combined	202	17.89	33.29	0	2.22			0.0 - 354.7	0.0 - 66.89
EPO u/ml	Female	108	13.99	38.83	0.3	0.45	-2.336	0.020	0.0 - 354.7	0.12 - 70.42
	Male	94	22.38	24.96	0.0	4.39			0.0 - 73.7	0.0 - 69.56
IL-6 pg/ml	Combined	202	5.52	28.46	0.0	0.03	-2.434		0.0 - 372.6	0.0 - 41.13
	Female	108	2.52	11.07	0.0	0.0		0.016	0.0 - 102.7	0.0 - 44.30
	Male	94	8.97	39.83	0.0	0.95			0.0 - 372.6	0.0 - 94.0
TNE «	Combined	202	10.42	44.85	0.0	1.25			0.0 - 525.2	0.0 - 59.61
ng/ml	Female	108	6.99	31.87	0.0	0.0	-3.851	< 0.001	0.0 - 325.1	0.0 - 59.80
P <i>B</i> /111	Male	94	14.37	56.12	0.0	4.55			0.0 - 525.2	0.0 - 126.9
CDD	Combined	202	11.02	11.44	0.0	7.25			0.0 - 48.7	0.0 - 39.86
CRP mg/ml	Female	108	14.6	12.9	0.0	11.70	-4.308	< 0.001	0.0 - 48.7	0.0 - 40.4
	Male	94	7.0	7.8	0.0	4.40			0.0 - 44.1	0.0 - 28.0

Table 4.4: Means medians, SD, modes, Ranges, 2.5 -97.5 percentiles Z-value, p-value of Epo and Proinflammatory cytokines

Epo = erythropoietin, IL-6 = Interleukin-6, TNF $\alpha$ = Tumor necrosis factor alpha, CRP= C-reactive protein. Wilcoxon ranksum test, p< 0.05, considered significant.

# 4.2 Effects of HIV infection in female and male subjects on the Haematological parameters

#### **4.2.1** Social demographics of HIV seropositive population

A total of 184 HIV seropositive patients comprising of 65 (35.3%) males and 119 (64.7%) females were recruited as cases for this study. Of these 155 (84.2%) were on antiretroviral therapy (ART) and the rest were ARV naïve. A total of 182 (98.9%) were non-smokers and 162 (88%) were non-alcohol drinkers. This study group had a mean age of  $35.3 \pm 9.74$  years and ranged from 19 to 59 years. The 155 ART respondents and the 29 ARV-naïve respondents were analyzed separately. The education background of the participants were as follows; Secondary school level 43.5%, tertiary 33.75, primary 22.3% and non- 0.5%. Their occupation included: formal employment 51.1%, business 41.3% studying 2.2% and house wife 5.4% (Table 4.5).

# Table 4.5: Social demographic characteristics of the HIV- seropositive group

Variable		Mean+ SD	Min- May	Frequency (%)
variable	_	Medil± 5D		Frequency (70)
Age (Vrs.)		353+974	19-59	
Education				
	None			1 (0.5%)
	Primary			41 (22.3%)
	Secondary			80 (43.5%)
	Tertiary			62 (33.7%)
Occupation				
	None			0 (0 %)
	House wife			10 (5.4%)
	Business			76 (41.3%)
	Employed			94 (51.1%)
	Student			4 (2.2%)
Smoking				
	Yes			2 (1.1%)
	No			182 (98.9%)
Alcohol				
	Yes			22 (12%)
	No			162 (88%)
ARV-treated				
	Yes			155 (84.2 %)
	No			29 (15.7%)

HIV-Infected (N = 184)

# 4. 2.2 Effects of HIV infection on morphological and blood cell values of female and male subjects

#### 4.2.2.1 Red blood cells

Erythrocytes from HIV negative study group (referents) were normal in size with diameters of almost the same sizes as those of the nuclei of small mature lymphocytes (Figure 4.9). Conversely, HIV positive female and male respondents displayed various types of anaemia such as microcytic hypochromic anaemia characterized by rouleaux formation and microcytic hypochromic red cells with, low HB levels (< 9.3 g/dl versus 9.5 g/dl females and males respectively), low MCH (< 23.7 g/dl versus < 22.8 g/dl), low MCHC (30.8 g/l versus< 29.2 g/dl) and low MCV (71.1 fl versus < 75.1 fl); The levels of this anaemia were in Antiretroviral treated (ART) females ranging from 4.4% to 15.4 % and spread across the CD4 groups. In Antiretroviral (ARV) – nave females the anaemia ranged from 2.6% to50 % but the distribution was concentrated more in CD4 groups of greater than 200 cell/mm<sup>3</sup>.In ART males the anaemia ranged from 2% to 50% and was sporadically spread in CD4 groups of cell counts of greater than 200 cells/mm<sup>3</sup>. ARV-naïve males did not demonstrate this type of anaemia at all (Figures<sup>1</sup> 4.10 and 4.11 and Tables<sup>1</sup> 4.6 – 4.8).

Moreover, macrocytic anaemia characterized by target cells and round macrocytes with high MCV (> 99.5fl versus 98 fl females and males respectively) and high MCH (> 34.3 g/l) was demonstrated. The anaemia levels ranged from 7.7% to 28.2% and was spread throughout ART females' study groups. In ARV-naïve females the anaemia levels ranged from 7.7% to 50 % with the highest levels seen in CD4 group of less than 200 cell/mm<sup>3</sup>. In both ART and ARV-naïve males, the anaemia was only demonstrated at base line and 3 months follow up stages in CD4 group of 500 or more cells/mm<sup>3</sup> (Figures 4.12 - 4.13 and Tables 4.9 - 12).





Figure 4.9: Normocyte (R) and a small Figure 4.10: Microcytic hypochromic cells (M) mature Lymphocyte (R1)



Figure 4.10: Rouleaux formation (R)



Figure 4.11: Target cells (T)



Figure 4.12: Round macrocyte (RM)

			CD4+ cells /mm <sup>3</sup>								
Parameter	$2.5^{\text{th}} - 97.5^{\text{th}}$	Abnormality	$< 200 (n=13)$ 200 – 499 (n =45) $\geq$ 500 (								
	noncontilog		F <sub>0</sub>	$F_1$	$F_2$	F <sub>0</sub>	$F_1$	$F_2$	F <sub>0</sub>	$F_1$	$F_2$
	percentiles		(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
RBC x 10 <sup>12</sup> /l	3.4 - 5.8 x 10 <sup>12</sup> /l	Erythropenia ( $< 3.4 \times 10^{12}$ /l)	0	0	0	11.1	22.2	0	0	0	0
HB g/dl	9.3- 17.6 g/dl	Anaemia (< 9.3 g/dl)	0	0	0	11.1	0	0	18.2	18.2	18.2
MCH g/l	23.7 - 34.3 g/dl	Hypochromia (< 23.7 g/l)	0	0	0	6.7	11.1	8.9	10.3	5.1	2.6
MCHC g/l	30.2 - 38.2	Hypochromia (<30.2. g/dl)	0	0	50	0	11.1	22.2	0	0	9.1

Table 4.6: Frequency of microcytic hypochromic anaemia in ART females

 $F_0 = Baseline, F_1 = 3 months, F_2 = 6 months$ 

# Table 4.7: Frequency of microcytic hypochromic anaemia in ARV- naive females

CD4+ cells /mm<sup>3</sup>

Parameter	$2.5^{\text{th}} - 97.5^{\text{th}}$	Abnormality	y < 200 (n= 2)			200 - 4	499 (n =	9)	≥ 500 (n = 11)		
	percentiles		F <sub>0</sub> (%)	F1 (%)	$F_2(\%)$	F <sub>0</sub> (%)	F1 (%)	F <sub>2</sub> (%)	F <sub>0</sub> (%)	F <sub>1</sub> (%)	F <sub>2</sub> (%)
RBC x 10 <sup>12</sup> /l	3.4 - 5.8 x 10 <sup>12</sup> /l	Erythropenia ( $< 3.410^{12}$ /l)	15.4	0	7.70%	4.40	13.30	6.70	15,4	15.40	7.70
HB	9.3- 17.6 g/dl	Anaemia (< 9.3 g/dl)	0	0	15.40	0	8.90	8.90	12.80	20.50	7.70
MCH g/l	23.7 - 34.3 g/l	Hypochromia (< 23.7 g/l)	0	0	0%	6.70	11.10	8.90	10.30	9.10	2.60
MCV fl	71.1 - 99.5 fl	Microcytosis (<71.1%)	0	0	0%	4.40	8.90	6.70	5.10	5.10	2.60
MCHC g/l	30.2 - 38.2g/l	Hypochromia (<30.2 g/l.)	7.70	7.70	0%	6.70	8.90	11.10	5.16	0	12.80

 $F_0 = Baseline, F_1 = 3 months, F_2 = 6 months$
		_		CD4							
Paramete r	$2.5^{\text{ th}}-97.5^{\text{ th}}$	Abnormality	<	200 (n=	5)	200	- 499 (n	=31)	≥	500 (n=	22)
	percentiles		F <sub>0</sub> (%)	F <sub>1</sub> (%)	F <sub>2</sub> (%)	F <sub>0</sub> (%)	F <sub>1</sub> (%)	F <sub>2</sub> (%)	F <sub>0</sub> (%)	F <sub>1</sub> (%)	F <sub>2</sub> (%)
HB g/dl	9.5 - 18.2 g/dl	Anaemia (< 9.3 g/dl)	0	0	0	0	0	50	0	0	0
MCH g/l	22.8 - 34.3 g/l	Hypochromia (<22.8 g/l)	0	0	0	0	0	0	50	50	50
MCV fl	75.1 - 98.0 fl	Microcytosis (<75.1 fl)	0	0	0	0	0	0	50	50	25
MCHC g/l	29.2 - 41.9 g/l	Hypochromia (<29.2g/l)	0	0	0	0	0	0	2	25	25

<b>Fable 4.8:</b> Frequency of microcytic	hypochromic	anaemia in ART	males
---	-------------	----------------	-------

 $F_0$  = Baseline,  $F_1$  = 3 months,  $F_2$ = 6 months

### Table 4.9: Frequency of macrocytic anaemia in ART females

CD4+ cells /mm <sup>3</sup>											
Parameter	$2.5^{\text{th}} - 97.5^{\text{th}}$	Abnormality	<	200 (n=	13)	200	- <b>499</b> (n	= 45)	≥	500 (n =	- 39)
	nercentiles		$\mathbf{F}_0$	$\mathbf{F}_1$	$F_2$	F <sub>0</sub>	$F_1$	$F_2$	F <sub>0</sub>	$F_1$	$F_2$
	percentiles		(%)	(%)	(%)	(%)	(%)	(%)	(%)	<del>(</del> %)	(%)
MCH	23.7 - 34.3	Hyperchromia (> 34.3 g/l)	0	23.1	7.7	17.8	15.6	13.3	15.4	20.5	28.2
MCV	71.1 - 99.5	Macrocytosis (> 99.5 fl)	7.7	23.1	15.4	20	15.6	22.2	20.5	25.6	28.2

			CD4+ cells /mm <sup>3</sup>										
Parameter	$2.5^{\text{ th}} - 97.5^{\text{ th}}$	Abnormality	< 2	200 (n=2	2)	200 -	- 499 (n	= 9 )	≥5	00 (n =	11)		
	percentiles		F <sub>0</sub> (%)	F <sub>1</sub> (%)	F <sub>2</sub> (%)	F <sub>0</sub> (%)	F <sub>1</sub> (%)	F <sub>2</sub> (%)	F <sub>0</sub> (%)	F <sub>1</sub> (%)	F <sub>2</sub> (%)		
MCH	23.7 - 34.3	Hyperchromia (> 34.3 g/l)	0	23.1	7.7	17.8	15.6	13.3	15.4	20.5	28.2		
MCV	71.1 - 99.5 fl	Macrocytosis (> 99.5 fl)	50	50	50	11.1	22.2	0	18.2	27.3	27.3		

### Table 4.10: Frequency of macrocytic anaemia in ARV-naïve females

 $F_0$  = Baseline,  $F_1$  = 3 months,  $F_2$ = 6 months

### Table 4.11: Frequency of macrocytic anaemia in ART males

CD4+ cells /mm<sup>3</sup>

Parameter	$2.5^{\text{ th}} - 97.5^{\text{ th}}$	Abnormality	<	< 200 (n= 5	5)	200 -	- 499 (n	= 31)	≥5	500 (n= 2	22)
	percentiles		F <sub>0</sub> (%)	$F_1(\%)$	F <sub>2</sub> (%)	F <sub>0</sub> (%)	F <sub>1</sub> (%)	F <sub>2</sub> (%)	F <sub>0</sub> (%)	F <sub>1</sub> (%)	F <sub>2</sub> (%)
MCV	75.1 - 98.0 fl	Macrocytosis (> 98 fl)	0	0	0	0	0	0	25	25	0

						CD4	+ cells /	mm <sup>3</sup>				
Parameter	$2.5^{\text{th}} - 97.5^{\text{th}}$	Abnormality	< 2	< 200 (n= 1) 200 - 499 (n = 2)						$\geq$ 500 (n = 4)		
	percentiles	percentiles		F <sub>1</sub> (%)	F <sub>2</sub> (%)	F <sub>0</sub> (%)	F <sub>1</sub> (%)	F <sub>2</sub> (%)	F <sub>0</sub> (%)	F <sub>1</sub> (%)	F <sub>2</sub> (%)	
MCV	75.1 - 98.0 fl	Macrocytosis (> 98 fl)	0	0	0	0	0	0	25	25	0	

## Table 4.12: Frequency of macrocytic anaemia in ARV-naive males

### 4.2.2.2 White blood cells and platelets

Neutrophilia (N >  $5.1 \times 10^{9}$ /l versus N > $5.3 \times 10^{9}$ /l in females and males respectively) and eosinophilia (E >  $0.6 \times 10^{9}$ / versus E > $.3 \times 10^{9}$ /l) with nuclear hypersegmentations were the main leucocyte abnormalities demonstrated. The distribution of these pathologies were as follows: neutrophilia 2.2% to 15.4% spreading across the CD4 groups and eosinophilia ranged from 5.1 to 11.1% across the groups in ART females. In ARV-naïve females sporadic absolute leucocytosis of 11.1% was demonstrated in CD4 less than 200 and between 200 and 499 cells /mm<sup>3</sup>. In ART males, neutrophil leucocytosis of 25% was only demonstrated in CD4 of 500 or more cells/mm<sup>3</sup> group (Figures: 4.15; 4.17 and Tables 16 – 18)

Thrombocytosis (P >446x10<sup>9</sup>/l versus P > 473x10<sup>9</sup>/l, females and males respectively) with giant platelets were also demonstrated in HIV positive respondents. Thrombocytosis ranging from 4.4% to 11.1% with highest levels in CD4 200-499 cells/mm<sup>3</sup> group was demonstrated in ART females. In ART males thrombocytosis of 25% was only demonstrated at baseline and 3 months follow up stages of CD4 group of 500 or more cells/mm<sup>3</sup> (Figure 4.18, tables 4.16 and 4.18). Other abnormalities demonstrated include: lymphopenia (L <  $1.6x10^{9}$ /l Versus L<  $1.0x10^{9}$ /l, females and males respectively) of up to 33% in ART females and up to 50% but sporadically distributed in ART males, neutropenia (N <  $1.1x10^{9}$ /l) ranging from 2.2% to 10.3% in ART females, monocytopenia (M< 0.2 x10<sup>9</sup>/l) of between 2.2% and 7.7% and thrombocytopenia (P <  $114x10^{9}$ /l) of between 2.5% and 5.1% in ART females (Tables 4.13- 4.17)



Figure 4.13: 3-lobe neutrophil normal (N1)



Figure 4.14: 6 lobed neutrophil, hypersegmenteded (N2)





Figure 4.15: Eosinophil with 3 nulear lobe (E) Figure 4.16: Hypersegmented eosinophil (E1)



Figure 4.17: Normal platelet (p) and a giant platelet (P1)

							CD4 o	cells/mm	3					
Parameter	$2.5^{\text{ th}} - 97.5^{\text{ th}}$	Abnormality	<	< 200 (n=13) 200 - 499 (n = 45)						$\geq$ 500 (n = 39)				
	norcontilos		$F_0$	$\mathbf{F}_1$	$F_2$	$F_0$	$F_1$	$F_2$	F <sub>0</sub>	$F_1$	$F_2$			
	percentiles		(%)	(%)	(%)	(%)	(%)	(%)	(%)	<del>(</del> %)	(%)			
WBC x 10 <sup>9</sup> /l	3.4 - 3.8x10 <sup>9</sup> /l	Leucocytosis (>3.8x10 <sup>9</sup> /l)	7.7	0	0	4.4	2.2	6.7	2.6	5.1	2.6			
N x 10 <sup>9</sup> /1	1.1 - 5.1x10 <sup>9</sup> /l	Neutrophilia (>5.1x10 <sup>9</sup> /l	15.4	7.7	0	4.4	2.2	6.7	5.1	0	0			
E x 10 <sup>9</sup> /l	0 - 0.6x10 <sup>9</sup> /1	Eosinophilia (> $0.6 \times 10^9$ /l)	0	7.7	7.7	8.9	8.9	11.1	7.7	5.1	5.1			
PLT x 10 <sup>9</sup> /l	114-446x10 <sup>9</sup> /l	Thrombocytosis > $446 \times 10^9$ /l)	7.7	0	0	11.1	4.4	11.1	7.7	5.1	2.6			

Table 4.13: Frequency of parameters leukocytosis and thrombocytosis in ART females

 $F_0 = Baseline$ ,  $F_1 = 3$  months,  $F_2 = 6$  months

### Table 4.14: Frequency of parameters leukocytosis and thrombocytosis in ARV-naive females

CD4+ cells /mm <sup>3</sup>											
Parameter	$2.5^{\text{th}} - 97.5^{\text{th}}$	Abnormality	< 2	200 (n=2	2)	200	<b>- 499 (r</b>	n = 9)	≥50	<b>)0 (n =</b>	11)
	percentiles		$F_0$	$F_1$	$F_2$	$F_0$	$F_1$	$F_2$	$F_0$	$F_1$	$F_2$
	-		(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
WBC x 10 <sup>9</sup> /l	3.4 - 3.8x 10 <sup>9</sup> /1	Leucocytosis (>3.8x 10 <sup>9</sup> /l)	11.1	0	0	0	0	11.1	0	0	0

	CD4 cells/mm <sup>3</sup>										
Parameter	$2.5^{\text{ th}}-97.5^{\text{ th}}$	Abnormality	< 2	00 (n=	5)	200 -	<b>499 (n</b>	=31)	≥5	00 (n =2	22)
	nercentiles		F <sub>0</sub>	$F_1$	$F_2$	$F_0$	$F_1$	$F_2$	$F_0$	$F_1$	$F_2$
	percentites		(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
W C x 10 <sup>9</sup> /l	2.3 - 7.8 x 10 <sup>9</sup> /1	Leucocytosis (>7.8 x 10 <sup>9</sup> /l)	0	0	0	0	0	0	25	0	25
N x 10 <sup>9</sup> /1	0.7 - 5.3 x 10 <sup>9</sup> /1	Neutrophilia (> $5.3 \times 10^9$ /l)	0	0	0	0	0	0	25	0	0
PLT x 10 <sup>9</sup> /1	92- 473 x 10 <sup>9</sup> /1	Thrombocytosis(>473x10 <sup>9</sup> /l)	0	0	0	0	0	0	25	25	0

 Table 4.15: Frequency of parameters leukocytosis and thrombocytosis in ART males

 $F_0 = Baseline, F_1 = 3 months, F_2 = 6 months$ 

### Table 4.16: Frequency of, Leucopenia, Neutropenia lymphopenia, monocytopenia and thrombocytopenia in ART Female

	CD4+ cells /mm <sup>3</sup>										
Parameter	$2.5^{\text{ th}} - 97.5^{\text{ th}}$	Abnormality	< 200	(n= 13)		200 -	499 (n =	= 45)	≥ 500	(n = 39	)
	norcontilos		$F_0$	$F_1$	$F_2$	$F_0$	$F_1$	$F_2$	F <sub>0</sub>	$F_1$	$F_2$
	percentiles		(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
WBC x 10 <sup>9</sup> /l	3.4 - 3.8 x 10 <sup>9</sup> /l	Leucopenia (< 3.4x 109/l)	15.4	15.4	15.4	4.4	2.2	4.40	10.3	7.7	7.70
N x 10 <sup>9</sup> /1	1.1 - 5.1 x 10 <sup>9</sup> /l	Neutropenia (< 1.1x 109/l)	0	7.70	7.7	2.2	8.7	2.2	7.7	7.7	10.3
L x 10 <sup>9</sup> /l	1.6 - 3.4 x 10 <sup>9</sup> /l	Lymphopenia (< 1.6x 109/l)	30.8	33.3	15.4	22.2	26.7	28.9	25.6	17.9	20.5
M x 10 <sup>9</sup> /1	0.2 - 1.5 x 10 <sup>9</sup> /l	Monocytopenia (< 0.2x 109/l)	7.70	7.7	0	2.2	6.7	2.2	2.6	0	2.6
PLT x 10 <sup>9</sup> /l	114 - 446 x 10 <sup>9/</sup> l	Thrombocytopenia (< 114 x 10 <sup>9</sup> /l)	0	0	0	2.2	0	2.5	5.1	0	0

						CD4	4 cells/m	1m <sup>3</sup>			
Parameter	$2.5^{\text{th}} - 97.5^{\text{th}}$	Abnormality	< 2	200 (n =	: 5)	200 - 499 (n = 31)			$\geq$ 500 (n = 22)		
	norcontilos		Fo	$F_1$	$F_2$	F <sub>0</sub>	$\mathbf{F}_1$	$F_2$	F <sub>0</sub>	$F_1$	$F_2$
	percentiles		(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Lx 10 <sup>9</sup> /1	1.0 - 4.1 x10 <sup>9</sup> /l	Lymphopenia (< 1.0x10 <sup>9</sup> /l)	0	0	0	0	0	50	0	0	25

## Table 4.17: Frequency of lympopenia in ART males

## 4. 2.3 Effects of HIV infection on levels of Haematological values of male and female Subjects

### 4.2.3.1 Baseline levels

Among the females significant decreases in haematological median values between the referents and ART respondents were demonstrated in: RBC in CD4 < 200 (Z=-2.386, p = 0.017) - and  $\geq$  500 (Z = -3.537, p< 0.001) cells/mm<sup>3</sup> groups, HB in CD4  $\geq$  500 (Z = -3.684, p< 0.001) cells/mm<sup>3</sup> group, L in CD4 200-499 (Z = -3.106, p = 0.002) - and  $\geq$  500 (Z = -2.913, p = 0.004) cells/mm<sup>3</sup> groups and in M in CD4  $\geq$ 500 (Z - -2.927, p = 0.003) cells/mm<sup>3</sup> group. Significant increases were demonstrated in E in CD4: < 200 (Z = -3.381, p = 0.001) -, 200-499 (-7.013, p < 0.001) - and  $\geq$  500 (Z = -6.020, p < 0.001) cell/mm<sup>3</sup> groups; in P in CD4 < 200 (Z = -3.311, p=0.001)-,  $\geq$  500 (Z = -3.958, p< 0.001) cells/mm<sup>3</sup> groups and in MCH in CD4 200-499 (Z = -2.893, p + 0.001) - and  $\geq$  500 (Z = -3.546, p<0.001) cells/mm<sup>3</sup> groups (Table 4.18).

Between referents and ARV – naïve females, significant increase were demonstrated in: WBC (Z= -2.386, p = 0.025), N (Z= -2.060, p = 0.039 and E (Z = -2.897, p = 0.004) in CD4 < 200 cells/mm<sup>3</sup> group; E (Z = -3.917, p < 0.001) and P (Z = -2.230, p = 0.026) in CD4 200 – 499 cells /mm<sup>3</sup> group). Significant decreases were demonstrated in: L (Z = -2.275, p = 0.023) in CD4 200-499 cells/mm<sup>3</sup> groups, HB (Z = -2.083, p = 0.037), WBC (Z = -2.996, p = 0.030) and L (Z = -4.848, p < 0.001) in CD4  $\geq$ 500 cells/mm<sup>3</sup> groups (Table 4.19)

CD4/mm <sup>3</sup>	Doromotor	Mean R	ank	Sum of R	anks	7 voluo	Asympt.
CD4/IIIII	1 al ameter	$F_N$	$F_0$	$F_N$	Fo	L- value	sig.(2-tailed
< 200	RBC	63.64	39.08	508.00	6873.00	-2.386	0.017*
	E	57.28	87.00	6129.00	1131.00	-3.381	0.001
	Р	57.34	91.42	6192.50	1188.50	-3.311	0.001
200 - 499	MCV	71.37	90.51	7708.00	4073.00	-2.435	0.015*
	MCH	70.31	93.06	7593.50	4187.50	-2.893	0.004
	L	84.17	59.79	9090.50	2690.50	-3.106	0.002*
	E	61.41	112.38	6571.00	5057.00	-7.013	< 0.001
$\geq$ 500	HB	81.77	52.47	8831.50	2046.50	-3.684	< 0.001*
	RBC	81.46	53.33	8798.00	2080.00	-3.537	< 0.001*
	MCV	66.52	94.72	7184.00	3694.00	-3.958	< 0.001
	MCH	72.42	78.38	7821.00	3057.00	-3.546	< 0.001
	L	80.14	57.00	8655.00	2223.00	-2.913	0.004*
	М	80.08	57.17	8648.50	2229.50	-2.927	0.003*
	Е	61.84	105.49	66.17.00	4114.00	-6.020	< 0.001
	Р	67.47	92.08	7287.00	3591.00	-3.093	0.003

 Table 4.18: Referents compared Female ART baseline parameters

 $F_N$  = Referents,  $F_0$  = Baseline. Wilcoxon rank-sum test , p< 0.05 is significant. \*Decrease

### Table 4.19: Referents compared Female ARV-naive baseline parameters

CD4/mm <sup>3</sup>	Parameter	Mean Rank		Sum of Ranks		Z- value	Asympt. sig.(2-
		$F_N$	F <sub>0</sub>	F <sub>N</sub>	F <sub>0</sub>	-	tailed
< 200	WBC	54.57	105.50	5894.00	211.00	-2.237	0.025
< 200	Ν	54.65	105.50	5902.00	203.00	-2.060	0.039
	E	54.00	108.50	5778.00	217.00	-2.897	0.004
200 - 499	L	61.06	34.33	6594.00	309.00	-2.275	0.023*
	E	65.46	94.67	5934.00	852.00	-3.917	< 0.001
	Р	56.98	83.22	6154.00	749.00	-2.230	0.026
$\geq$ 500	HB	62.10	39.36	6707.00	433.00	-2.083	0.037*
	WBC	63.02	30.32	6806.50	333.50	-2.996	0.030*
	L	64.88	12.05	7007.50	132.50	-4.848	< 0.001*
	М	62.33	37.14	6731.50	408.50	-2.339	0.019*

 $F_N$  = Referents,  $F_0$  = Baseline. Wilcoxon rank-sum test, p< 0.05 is significant. \*Decrease

Among males Significant increases in haematological values between referents and ART respondents were demonstrated in: WBC (Z = -2.413, p = 0.016) in CD4 < 200 -, MCV

(Z = -3.136, p = 0.002), MCH (Z = -2.264, p = 0.024) and N (Z = -2.673, p = 0.008) in CD4 200 – 499 cells/mm<sup>3</sup>groups. Conversely, decreases were demonstrated in RBC in: CD4 < 200 (Z = -1.999, p = 0.046) -, 200- 499 (Z = -2.960, p = 0.0016)- and  $\geq$ 500 (Z = -3.118, p = 0.002) cells/mm<sup>3</sup> groups (Table 4.20). In ARV-naive respondents significant increases were demonstrated in P in: CD4< 200 (Z = -2.244, p = 0.025) -, 200-499 (Z = -2.2042, p = 0.022) – and  $\geq$ 500 (Z = -2.244, p = 0.025) cells/mm<sup>3</sup> groups; while decreases were demonstrated in RBC (Z = -2.983, p = 0.003) in CD4 < 200- , L (-2.042, p = 0.041) in CD4 200-499-, HB (Z = -2.060, p = 0.046) and RBC (Z = -2.983, p = 0.0030) in CD4  $\geq$ 500 cells/mm<sup>3</sup> groups (Table 4.21).

CD4/mm <sup>3</sup>	Parameter	Mean R	Mean Rank Sum of Ranks		Z- value	Asympt. sig.(2- tailed)	
		F <sub>N</sub>	F <sub>0</sub>	$F_N$	$F_0$		
< 200	RBC	51.33	25.00	4825.00	125.00	-1.999	0.046*
	WBC	43.39	80.20	4549.00	401.00	-2.413	0.016
200 - 499	RBC	68.51	46.31	6539.50	1435.50	-2.960	0.003*
	MCV	57.16	80.69	5373.50	2501.50	-3.136	0.002
	MCH	58.79	75.77	5526.00	2349.00	-2.264	0.024
	Ν	58.03	78.06	5455.00	2420.00	-2.673	0.008
	L	68.99	46.82	6485.50	1389.50	-3.226	0.001*
	PLTs	53.85	90.76	5061.50	2813.50	-4.920	< 0.001
$\geq$ 500	RBC	63.21	38.39	5941.50	844.50	-3.118	0.002*
	L	61.85	44.20	5813.50	972.50	-2.218	0.027*
		_					

Table 4.20: Referents compared with ART male baseline parameters

 $F_N$  = Referents,  $F_0$  = Baseline. Wilcoxon rank-sum test, p< 0.05 is significant. \*Decrease

<b>Table 4.21: F</b>	Referents com	oared ARV- naïve	e male baseline	parameters
----------------------	---------------	------------------	-----------------	------------

CD4/mm <sup>3</sup>	Donomotor	Mean Rank		Sum of Ranks		Z-	Asympt.
CD4/IIIII	rarameter	$F_N$	F <sub>0</sub>	$F_N$	F <sub>0</sub>	value	sig.(2-tailed
< 200	RBC	51.27	8.00	4819.00	32.00	-2.983	0.003*
	PLTs	47.53	92.00	4468.00	92.00	-2.244	0.025
200 - 499	L	49.35	8.75	4638.50	17.50	-2.042	0.041*
	PLTs	47.55	93.00	4470.00	186.00	-2.042	0.022
> 500	HB	50.19	21.38	4667.50	85.50	-2.006	0.045*
	RBC	51.27	8.00	4819.00	32.00	-2.983	0.003*
	PLTs	48.32	77.25	4542.00	309.00	-2.244	0.025

 $F_N = \text{Referents}, F_0 = \text{Baseline}$ . Wilcoxon rank-sum test., p< 0.05 is significant. \*Decrease

### 4.2.3.2 Longitudinal levels

During follow up significant increases in ART female respondents' haematological parameters were demonstrated in: RBC between 3 months and baseline (Z = -2.957, p =0.003), 6 months and baseline (Z = -3.184, p = 0.001), WBC between 6 months and baseline (z = -2.245, p = 0.025) and N between 6 months and baseline (Z = -2.943, p = 0.003) in CD4 < 200 cells/mm<sup>3</sup> group; HB between 3 months and baseline (Z = -2.967, p = 0.003) 6 months and baseline (Z = -3.184, p = 0.001), N between 6 months and baseline (Z = -2.440, p = 0.015); L between 3 months and baseline (Z = -2.092, p = 0.036) and P between 3 months and baseline (Z = -2.498, p = 0.013) in CD4 200-499 cells/mm<sup>3</sup>. In CD4  $\geq$ 500 cells/mm<sup>3</sup> group, the increases were demonstrated in WBC (Z = -2.054, p< 0.001) between 6 months and baseline, N (Z = -3.373, p = 0.001) between 6 months and baseline and P (Z = -2.154, p = 0.031) between 3 months and baseline. Significant decreases were demonstrated in: RBC (Z = -2.955, p = 0.003) between 6 and 3 months in CD4 < 200 cell/mm<sup>3</sup> group, RBC between 6 months and baseline (Z = -2.469, p = 0.0014) between 6 and 3 months (-3.006, p = 0.003) and N (Z = -2.277, p = 0.023) between 3 months and baseline in CD4 200 – 499 cells/mm<sup>3</sup> group. In CD4  $\geq$  500 cells/mm<sup>3</sup> group decreases were demonstrated in HB (Z = -2.955, p = 0.003) between 6 and 3 months, MCHC (Z = -2.402, p = 0.016) between 3 months and baseline and N (Z = -2.277, p= 0.026) between 3 months and baseline (Table 4.22)

In ARV-naïve female respondents significant increases in haematological parameters were demonstrated only in MCH (Z = -2.049, p = 0.040) and M (Z = -2.126, p = 0.036) between 3 months and baseline in CD4  $\geq$  500 cells/mm<sup>3</sup> group (table 4.23)

$CD1/mm^3$	Doromotor	Mean	Sum of	7 voluo	Asympt.
CD4/IIIII	I al alletel	Rank	Ranks	L- value	sig.(2-tailed)
< 200	$RBC F_1 - RBC F_0$	14.70	147.00	2.957	0.003
< 200	$RBC F_2 - RBC F_0$	15.50	217.00	-3.184	0.001
	$RBC F_2 - RBC F_1$	15.12	207.00	-2.955	0.003*
	WBC $F_2 - WBC F_0$	3.50	10.50	-2.245	0.025
	$N\;F_2-N\;F_0$	1.50	1.50	-2.943	0.003
200 - 499	RBC $F_2 - RBC F_0$	23.19	603.00	-2.469	0.0014*
	RBC $F_2 - RBC F_1$	21.12	442.50	-3.006	0.003*
	$HB F_1 - HBF_0$	16.25	227.50	-2.967	0.003
	$HB F_2 - HBF_0$	18.12	235.50	-3.184	0.001
	N $F_1$ -N $F_0$	22.73	269.50	-2.277	0.023*
	$N F_2 - N F_0$	19.36	271.00	-2.440	0.015
	$L \ F_1 - L \ F_0$	23.07	646.00	-2.092	0.036
	$P \ F_1 - P \ F_0$	21.62	281.00	-2.498	0.013
≥ 500	$HB \ F_2 - HBF_1$	19.77	474.50	-2.955	0.003*
	MCHC F <sub>1</sub> – MCHC F <sub>0</sub>	2.00	6.00	-2.402	0.016*
	WBC F2-WBC F0	19.08	229.00	-2.054	< 0.001
	$N F_1 - N F_0$	19.96	499.00	-2.277	0.026*
	N F <sub>2</sub> - N F <sub>0</sub>	18.50	148.50	-3.373	0.001
	$\mathbf{P} \mathbf{F}_1 - \mathbf{P} \mathbf{F}_0$	24.67	22.00	-2.154	0.031

Table 4.22: Differences in ART female haematological parameters during follow up

 $F_{0=}$ Baseline,  $F_{1=3}$  months,  $F_{2=}$  6 months. \* Decrease. Wilcoxon rank-sum test, p< 0.05 is significant (2-tailed test)

 Table 4.23: Differences in ARV-naive female haematological parameters during follow up

CD4/mm <sup>3</sup>	Parameter	Mean Rank	Sum of Ranks	Z- value	Asympt. sig.(2-tailed)
≥ 500	MCH F <sub>1</sub> - MCH F <sub>0</sub>	6.22	56.00	-2.049	0.040
	M F <sub>1</sub> - M F <sub>0</sub>	4.64	32.50	-2.126	0.036

 $F_{0=}$ Baseline,  $F_{1}$ =3 months,  $F_{2}$ =6 months. Wilcoxon rank-sum test, p<0.05 is significant (2-tailed test)

In ART male respondents significant increases in haematological median values were demonstrated in WBC (Z = -2.032, p = 0.040) between 3 months and baseline in CD4 < 200 cells/mm<sup>3</sup> group; in N (Z = -2.109, p = 0.035) between 6 months and baseline, L (Z = -2.958, p = 0.003) between 3 months and baseline and P (Z = -2.023, p = 0.043) between 6 months and baseline in CD4 200 - 499 cells/mm<sup>3</sup> group. In CD4  $\geq$  500 cells/mm<sup>3</sup> group significant increases were demonstrated in WBC (Z = -2.260, p = 0.024) between 6 months and baseline, N between 6 months and baseline (Z = -2.235, p = 0.020) between 6 and 3 months (Z = -3.138, p = 0.002), M between 3 months and baseline and between 6 months and baseline (Z = -2.892, p = 0.004). Significant decreases were demonstrated in: MCV (Z = -2.294, p = 0.022) between 6 and 3 months and in MCHC (Z = -2.295, p = 0.026) between 6 months and baseline both in CD4 200 – 499 ells/mm<sup>3</sup> group. In CD4  $\geq$  500 cells/mm<sup>3</sup> group decreases were demonstrated in HB between 6 months and baseline (Z =-2.469, p = 0.014) and between 6 and 3 months (Z = -3.006, p = 0.003), P between 3 months and baseline (Z = -2.792, p = 0.005) and between 6 months and baseline (Z = -2.197, p = 0.033) (Table 4.24).

$CD4/mm^3$	Donomotor	Mean	Sum of	7 voluo	Asympt.
CD4/IIIII	Parameter	Rank	Ranks	<b>Z</b> - value	sig.(2-tailed)
< 200	WBC F <sub>1</sub> - WBC F <sub>0</sub>	0.00	0.00	-2.032	0.040
< 200					
200 - 499	MCV F2 - MCV F1	18.21	255.00	-2.294	0.022*
	MCHC F <sub>2</sub> - MCHC F <sub>0</sub>	11.21	134.50	-2.225	0.026*
	N F <sub>2</sub> - N F <sub>0</sub>	12.28	110.5	-2.109	0.035
	$L F_1 - LF_0$	13.57	312.00	-2.958	0.003
	$P F_2 - P F_0$	0.00	0.00	-2.023	0.043
≥ 500	HB F <sub>2</sub> - HBF <sub>0</sub>	7.21	50.50	-2.469	0.014*
	HB $F_2$ - HBF <sub>1</sub>	8.50	34.00	-3.006	0.003*
	WBC F2 -WBC F0	7.21	50.50	-2.260	0.024
	N F <sub>2</sub> - N F <sub>0</sub>	12.33	37.00	-2.235	0.020
	$N F_2 - N F_1$	8.50	22.50	-3.138	0.002
	M F <sub>1</sub> - M F <sub>0</sub>	8.67	104.90	-2.529	0.011
	M F 2 - M F0	9.13	137.00	-2.892	0.004
	P F <sub>1</sub> - P F <sub>0</sub>	6.75	40.50	-2.792	0.005*
	P F <sub>2</sub> - P F <sub>0</sub>	10.12	61.00	-2.127	0.033*

Table 4.24: Differences in ART male haematological parameters during follow up

 $F_0$  = Baseline,  $F_1$ =3 months,  $F_2$ = 6 months. \* Decrease. Wilcoxon rank-sum test , p< 0.05 is significant (2-tailed test)

### 4.2.3.3 Levels in antiretroviral treated and untreated respondents

Significant increases in haematological median values between ART and ARV-naïve female respondents were demonstrated in E at baseline (Z = -2.246, p = 0.025), at 3 months (Z = -2.302, p =0.021) and at 6 months (Z = -2.291, p = 0.022) in CD4 < 200 cells/mm<sup>3</sup> group. In CD4  $\geq$  500 cells/mm<sup>3</sup> group the increases were demonstrated in RBC at baseline (Z = -2.049, p = 0.041) and at 3 months (Z = -2.313, p = 0.021); while decreases were demonstrated in WBC (Z = -1.980, p = 0.048) and L (Z = -2.089, p = 0.037) at baseline (table 4.25). No significant differences in haematological parameters between ART and ARV-naïve male respondents were demonstrated.

CD4		Mear	Mean Rank		Sum of Ranks		Asymptotic	
cells/mm <sup>3</sup>	Parameter	ART	ARV- naive ART		ARV- naive	value	sig.(2-tailed)	
< 200	E F <sub>0</sub>	7.00	14.50	91.00	29.00	-2.246	0.025	
	$E F_1$	7.00	14.50	91.00	29.00	-2.302	0.021	
	$E F_2$	7.00	14.50	91.00	29.00	-2.291	0.022	
≥ 500	RBC F <sub>0</sub>	23.27	33.41	907.50	367.50	-2.049	0.041	
	RBC F <sub>1</sub>	22.97	34.45	896.00	379.00	-2.313	0.021	
	WBC F <sub>0</sub>	27.67	17.82	1079.00	196.00	-1.980	0.048*	
	L F <sub>0</sub>	28.74	17.41	1083.50	191.50	-2.089	0.037*	

 Table 4.25:
 Significant differences in haematological parameters between ART and ARV-naïve females.

 $F_0$  = Baseline,  $F_1$ =3 months,  $F_2$ = 6 months. \* Decrease. Wilcoxon rank-sum test, p< 0.05 is significant (2-tailed test).

### 4.2.3.4 Levels within CD4 groups

Within the CD4 groups significant difference in haematological parameters were demonstrated in ART female respondents in M ( $X^2 = 6.222$ , p = 0.045) at baseline (Table 4.26). In ARV-naïve females the differences were demonstrated in: WBC ( $X^2 = 11.172$ , p = 0.004) and N ( $X^2 = 8.316$ , p = 0.0016) at baseline, WBC ( $X^2 = 6.635$ , p = 0.036) at 3 months, WBC ( $X^2 = 6.148$ , p = 0.046) and E ( $X^2 = 9.644$ , p = 0.008) at 6 months (Table 4.27).

 Table 4.26: Within group changes in haematological parameters in ART females

Parameter	Chi-square (X <sup>2</sup> )	Df	Asymptotic sig.(2-tailed)
M F <sub>0</sub>	6.222	2	0.045

Kruskal – Wallis test, p < 0.05 is significant.  $F_0$  = Baseline

Parameter	Chi-square (X <sup>2</sup> )	Df	Asymptotic sig.(2-tailed)
WBC F <sub>0</sub>	11.172	2	0.004
$WBC F_1$	6.635	2	0.036
WBC F <sub>2</sub>	6.148	2	0.046
N $F_0$	8.316	2	0.016
$E F_2$	9.644	2	0.008

Table 4.27: Within group changes in haematological parameters in ARV-naïve females

Kruskal –Wallis test, p<0.05 is significant.  $F_{0\,=}\,Baseline,\,F_{1}{=}3$  months,  $F_{2}{=}\,6$  months

In males differences in haematological parameters within CD4 groups were demonstrated in ART males in each of MCH ( $X^2 = 6.472$ , p = 0.039), MCHC ( $X^2 = 9.616$ , p = 0.008) at 6 months, N ( $X^2 = 6.304$ , p = 0.043) and P ( $X^2 = 10.682$ , p = 0.005) at 6 months (Table 4.28)

### Table 4.28: Within group changes in haematological parameters in ART males

Parameter	Chi-square (X <sup>2</sup> )	Df	Asymptotic sig.(2-tailed)
MCH F <sub>2</sub>	6.478	2	0.039
MCHC F <sub>2</sub>	9.616	2	0.008
N F <sub>0</sub>	6.304	2	0.043
$\mathbf{P} \mathbf{F}_0$	10.682	2	0.005

Kruskal – Wallis test, p < 0.05 is significant.  $F_0 =$  Baseline,  $F_2 = 6$  months

# 4.3 Effects of HIV infection in male and female subjects on the levels of Biochemical Parameters

### 4.3.1 Baseline levels

In females significant increases in Biochemical median values between referents and ART females' baseline parameters were demonstrated in: ALT (Z = -3.352, p = 0.001), GGT (Z

= -2.663, p = 0.008), ALP (Z = -2.549, p = 0.001) and ALB (Z = -2.922, p = 0.003) in CD4 < 200 cells/mm<sup>3</sup> group. In CD4 200 – 499 cells/mm<sup>3</sup> group the increases were demonstrated in: AST (Z= -5.547, p < 0.001), ALT (Z = -6.312, p < 0.001), GGT (Z = -5.580, p< 0.001), ALP (Z = -3.130, p = 0.002), and ABL (Z = -4.365, p < 0.001). In CD4 ≥ 500 cells/mm<sup>3</sup> group the increases were demonstrated in: AST (Z = -3.322, p = 0.001), ALT (Z = -5.336, p < 0.001) and GGT (Z = -4.567, p < 0.001). Conversely significant decreases were demonstrated in: T.Bil (Z = -5.144, p< 0.001), D.Bil (Z = -4.919, p < 0.001) T.PRT (Z = -2.445, p = 0.004) and CRT (Z = -4.965, p< 0.001) in CD4 < 200 cells/mm<sup>3</sup>. In CD4 200 – 499 cells/mm<sup>3</sup> group significant decreases were demonstrated in: T.Bil (Z = -5.809, p < 0.001), T.PRT (Z = -5.064, p < 0.001) and UREA (Z = -2.283, p = 0.023); while in CD4 ≥500 cells/mm<sup>3</sup> group, the decreases were demonstrated in: T.Bil (Z = -5.232, p < 0.001), D.Bil (Z = -5.232, p < 0.001), D.Bil (Z = -5.232, p < 0.001), D.Bil (Z = -5.064, p < 0.001) and UREA (Z = -2.283, p = 0.023); while in CD4 ≥500 cells/mm<sup>3</sup> group, the decreases were demonstrated in: T.Bil (Z = -5.232, p < 0.001), D.Bil (Z = -5.232, p < 0.001), D.Bil (Z = -5.064, p < 0.005) and T.PRT (Z = -5.061, p = <0.001) (Table 4.29).

In ARV- naïve females significant increases above referents median values were demonstrated in: GGT (Z = -2.272, p = 0.022) in CD4 < 200 cells/mm<sup>3</sup> group, AST (Z = -2.223, p = 0.026), ALT (Z = -3.196, p = 0.001), GGT (Z = -2.610, p = 0.009) and ALP (Z = -3.299, p = 0.001) in CD4 200 - 499 cells /mm<sup>3</sup> group and AST (Z = -2.049, p= 0.040 , ALT (Z = -4.138, p < 0.001 and GGT (Z = -3.993, p < 0.001)) in CD4  $\ge$  500 cells/mm<sup>3</sup> group. Contrarily significant decreases were demonstrated in: AST (Z = -2.420, p = 0.016) in CD4 < 200 cells/mm<sup>3</sup> group, T.Bil (Z = -3.769, P < 0.001), D. Bil (Z = -3.441, p = 0.001) and T.PRT (Z = -3.265, p = 0.001) in CD4 200-499 cells/mm<sup>3</sup> group and T.PRT (Z = -3.516, p =< 0.001) in CD4  $\ge$  500 cells/mm<sup>3</sup> group (Table 4.30).

CD4/mm <sup>3</sup>	Parameter	Mear	Mean Rank Sum of Ranks Z-value		Sum of Ranks		Asymptotic
		Fn	Fo	F <sub>N</sub>	Fo		Sig. 2-tailed
. 200	ALT	57.30	91.77	6188.00	1193.00	-3.352	0.001
< 200	GGT	58.06	85.46	6270.00	1111.00	-2.663	0.008
	ALP	58.18	84.42	6283.50	1077.50	-2.549	0.001
	T.BIL	66.69	13.73	7202.50	178.50	-5.144	< 0.001*
	D.BIL	65.70	13.71	7095.50	164.50	-4.919	< 0.001*
	T.PRT	63.70	35.54	6870.00	501.00	-2.445	0.014*
	ALB	57.78	87.77	62.40	1141.00	-2.922	0.003
	CRT	64.28	33.73	6942.50	438.50	-4.965	< 0.001*
200 - 499	AST	64.19	107.76	6832.00	4849.00	-5.547	< 0.001
	ALT	62.42	112.00	6741.00	504000	-6.312	< 0.001
	GGT	64.10	107.75	6923.00	4858.00	-5.580	< 0.001
	ALP	69.79	94.31	7537.00	4244.00	-3.120	0.002
	T.BIL	94.07	36.03	10159.50	1621.50	-7.388	< 0.001*
	D.BIL	90.38	44.89	9761.00	2020.00	-5.809	< 0.001*
	T.PRT	88.70	48.91	9590.00	2201.00	-5.064	< 0.001*
	ALB	66.93	101.17	7228.50	4552.50	-4.365	< 0.001
	UREA	82.27	64.34	8885.50	2895.50	-2.283	0.023*
≥ 500	AST	67.00	93.38	7236.00	3642.00	-3.322	0.001
	ALT	62.75	105.15	6777.00	4101.00	-5.336	< 0.001
	GGT	64.37	100.68	6951.50	3926.50	-4.567	< 0.001
	T.BIL	85.08	43.31	9189.00	1689.00	-5.252	< 0.001*
	D.BIL	79.86	57.77	86250	2253.00	-2.780	0.005*
	T.PRT	84.68	44.44	9145.00	1733.00	-5.061	< 0.001*

 Table 4.29: Referents compared with female ART baseline Biochemical

 $F_{\rm N}$  = Referents,  $F_0$  = Baseline Wilcoxon rank-sum test, p< 0.05 is significant. \* Decrease

## Table 4.30: Referents compared with ARV-naïve female baseline Biochemical parameters

	Parameter	Mean	Rank	Sum of	Ranks	Z-value	Asymptotic
		FN	Fo	F <sub>N</sub>	Fo	-	Sig. 2-tailed
< 200	AST	56.50	1.50	6102.00	3.00	-2.420	0.016*
	GGT	54.56	106.25	5892.50	212.50	-2.272	0.022
200 - 499	AST	56.99	83.11	6155.00	748.00	-2.223	0.026
	ALT	56.11	93.67	6060.00	843.00	-3.196	0.001
	GGT	56.64	87.33	6117.00	786.00	-2.610	0.009
	ALP	56.01	94.83	6049.50	853.50	-3.299	0.001
	T.BIL	62.41	18.06	6740.50	162.50	-3.769	< 0.001*
	D.BIL	62.11	21.67	6708.00	195.00	-3.441	0.001*
	T.PRT	61.95	23.56	6691.00	252.00	-3.265	0.001*
≥ 500	AST	57.94	80.27	6257.00	883.00	-2.049	0.040
	ALT	55.83	100.95	6029.50	1110.50	-4.138	< 0.001
	GGT	55.97	98.55	6045.00	1095.00	-3.993	< 0.001
	T.PRT	63.55	25.18	6863.00	277.00	-3.516	< 0.001*

 $F_N$  = Referents,  $F_0$  = Baseline. Wilcoxon rank-sum test, p< 0.05 is significant. \* Decrease

Among the male respondents significant increases in Biochemistry median values between the referents and ART baseline values were demonstrated in: GGT (Z = -2.318, p = 0.012) and T.PRT (Z = -2.008, p = 0.045) in CD4 < 200 cells/mm<sup>3</sup> group; AST (Z = 2.990, p = 0.003) and GGT (Z = -3.929, p = 0.001) in CD4 200 – 499 cells/mm<sup>3</sup> group and ALT (Z = -2.379, p = 0.017), GGT (Z = -2.199, p = 0.028) and T.PRT (Z = -2.122, p = 0.034) in CD4  $\geq$  500 cells /mm<sup>3</sup> group. On the other hand decreases were demonstrated in T.Bil (Z = -3.412, p = 0.001) and D.Bil (Z = 12.117, p = 0.0034) in CD4 < 200 cells/mm<sup>3</sup> group; T.Bil (Z = -3.790, p < 0.001) and UREA (Z = -3.635, p < 0.001) in 200 – 499 cells /mm<sup>3</sup> group and ALP (Z = -3.170, p = 0.002), T.Bil (Z = -2.940, p = 0.003) and D.Bil (Z = -2.842, p = 0.004) in  $\geq$  500 cells /mm<sup>3</sup> group (Table 4.31).

In ARV – naïve males significant increases were demonstrated in: ALB (Z = -2.084, p = 0.037) in CD4 200 - 499 cells/mm<sup>3</sup> group, and ALT (Z = -1.995, p = 0.046) in CD4  $\ge$  500 cells /mm<sup>3</sup> group; while decreases were demonstrated in: T.Bil (Z = - 2.193, p = 0.028) in CD4 200 - 499 cells/mm<sup>3</sup> group, T.Bil (Z = - 3.016, p = 0.003) and D. Bill (Z = -3.061, p = 0.002) in CD4  $\ge$  500 cells /mm<sup>3</sup> group (Table 4.32).

CD4/mm <sup>3</sup>	_	Mean	Rank	Sum of	f Ranks	_	Asymptotic
	Parameter	$\mathbf{F}_{\mathbf{N}}$	Fo	$\mathbf{F}_{\mathbf{N}}$	Fo	Z-value	Sig.( 2- tailed)
< 200	GGT	48.32	81.50	4542.50	407.50	-2.518	0.012
	T.BIL	52.27	7.30	4913.50	36.50	-3.412	0.001*
	D.BIL	51.41	23.50	4832.50	117.50	-2.117	0.034*
	T.PRT	48.66	75.10	4574.50	375.50	-2.008	0.045
200 - 499	AST	57.44	79.85	5399.50	2475.50	-2.990	0.003
	GGT	55.69	85.16	5235,00	2640,00	-3.929	< 0.001
	T.BIL	70.05	41.61	6585,00	1290,00	-3.790	< 0.001*
	UREA	66.76	42.50	6557.50	1371.50	-3.635	< 0.001*
≥ 500	ALT	54.91	73.84	5161.50	1624.50	-2.379	0.017
	GGT	55.18	72.68	5187,00	1599,00	-2.199	0.028
	ALP	63.29	38.05	5948,00	837,00	-3.170	0.002*
	T.BIL	62.94	39.52	5916.50	869.50	-2.940	0.003*
	D.BIL	62.79	40.16	5902.50	883.50	-2.842	0.004*
	T.PRT	55.30	72.18	5198,00	1588,00	-2.122	0.034

 Table 4.31: Referents compared with ART male baseline Biochemical parameters

 $F_N$  = Referents,  $F_0$  = Baseline. Wilcoxon rank-sum test, p< 0.05 is significant. \* Decrease

# Table 4.32: Referents compared with ARV-naïve male baseline Biochemical parameters

CD4/mm <sup>3</sup>	Parameter	Mean	Rank	Sum of Ranks		Sum of Ranks		Z-value	Asymptotic
		<b>F</b> <sub>N</sub>	F <sub>0</sub>	$\mathbf{F}_{\mathbf{N}}$	Fo		Sig. 2-tailed		
200 - 499	T.BIL	49.41	5.75	4544.50	11.50	-2.193	0.028*		
	ALB	47.64	89.00	4478.00	178.00	-2.084	0.037		
≥ 500	ALT	48.32	77.25	4542.00	309.00	-1.995	0.046		
	T.BIL	51.29	7.50	4821.00	30.00	-3.016	0.003*		
	D.BIL	51.31	6.88	4823.50	27.50	-3.061	0.002*		

 $F_N$  = Referents,  $F_0$  = Baseline. Wilcoxon rank-sum test, p< 0.05 is significant. \* Decrease

### 4.3.2 Longitudinal levels

\_

During the 6 months period, significant increases in Biochemical parameters in ART female respondents were demonstrated in: AST (Z = -2.065, p = 0.039) between 6 and 3 months, ALP between 3 months and baseline (Z = -2.341, p = 0.019) and between 6 months and baseline (Z = -2.412, p = 0.016), T.PRT (Z = -2.203, p = 0.028) between 3

months and baseline, ALB between 3 months and baseline (Z = -2.598, p = 0.009) and between 6 months and baseline (Z = -2.693, p = 0.007) and UREA (Z = -2.062, p = 0.039) between 6 months and baseline in CD4 < 200 cells/mm<sup>3</sup> group. In CD4 200 - 499 cells/mm<sup>3</sup> increases were demonstrated in; AST between 6 months and baseline (Z = -3.327, p = 0.001) and between 6 and 3 months (Z = 3.863, p < 0.001), GGT between 6 months and baseline (Z = -2.038, p = 0.042) and between 6 and 3 months (Z = -2.884, p =(0.004), ALP between 3 months and baseline (Z = -3.393, p = 0.001) and between 6 months and baseline (Z = -3.280, p = 0.001), T.PRT (Z = -2.796, p = 0.005) between 6 months and baseline, ALB (-3.277, p = 0.001) between 6 months and baseline and UREA between 3 months and baseline (Z = -2.097, p = 0.030) and between 6 months and baseline (Z = -2.097, p = 0.030) 2.166, p = 0.030). In CD4  $\geq$  500 cells /mm<sup>3</sup> group significant increases were demonstrated in: AST between 6 months and baseline (Z = -2.185, p = 0.029) and between 6 and 3 months (Z -2.169, p = 0.030), GGT between baseline and 3 months (Z = -3.644, p < 0.001) and between 6 and 3 months (Z = -3.249, p = 0.001), ALP (Z = -2.533, p = 0.011) between 6 and baseline, D.Bil (Z = -2.281, p = 0.022), T.PRT between 3 months and baseline (Z = -2.281, p = 0.022), T.PRT between 3 months and baseline (Z = -2.281, p = 0.022), T.PRT between 3 months and baseline (Z = -2.281, p = 0.022), T.PRT between 3 months and baseline (Z = -2.281, p = 0.022), T.PRT between 3 months and baseline (Z = -2.281, p = 0.022), T.PRT between 3 months and baseline (Z = -2.281, p = 0.022), T.PRT between 3 months and baseline (Z = -2.281, p = 0.022), T.PRT between 3 months and baseline (Z = -2.281, p = 0.022), T.PRT between 3 months and baseline (Z = -2.281, p = 0.022), T.PRT between 3 months and baseline (Z = -2.281, p = 0.022), T.PRT between 3 months and baseline (Z = -2.281, p = 0.022), T.PRT between 3 months and baseline (Z = -2.281, p = 0.022), T.PRT between 3 months and baseline (Z = -2.281, p = 0.022), T.PRT between 3 months and baseline (Z = -2.281, p = 0.022), T.PRT between 3 months and baseline (Z = -2.281, p = 0.022). -3.087, p = 0.002) and between 6 and 3 months (Z = -3.707, p < 0.001) and CRT (Z = -3.707, p < 0.001) 2.331, p = 0.020) between 6 months and baseline.

Moreover, significant decreases in CD4 < 200 cells/mm<sup>3</sup> group were demonstrated in: AST (Z = -2.707, p = 0.007) between 3 months and baseline, T.Bil between 3 months and baseline (Z = -2.621, p < 0.001) and between 6 months and baseline (Z = -2.903, p = 0.004), D. Bill between 3 months and baseline (Z = -2.366, p = 0.018) and between 6 months and baseline (Z = -2.393, p = 0.016), CRT between 3 months and baseline (Z = -2.900, p = 0.004) and between 6 months and baseline (Z = -2.353, p = 0.019). In CD4 200 - 499 cells/mm<sup>3</sup> group the decreases were demonstrated in: T.Bil between 3months and baseline (Z = -4.498, p < 0.001) and between 6 months and baseline (Z = -4.081, p =<0.001) and CRT (Z = -2.150, p = 0.032) between 3 months and baseline. In CD4  $\geq$  500 cells /mm<sup>3</sup> group decreases were demonstrated in ALB (Z = -3.353, p = 0.001) between 3 months and baseline (Table 4.33).

In ARV-naïve females significant increases were demonstrated in: D.Bil between 3 months and baseline (Z= -2.393, p = 0.022) and between 6 months and baseline (Z = -2.312, p = 0.021), T.PRT (Z = -2.105, p = 0.035) between 6 and 3 months in CD4  $\geq$  500 cells/mm<sup>3</sup> group; while significant decreases were demonstrated in T.Bil (Z = -1.836, p = 0.043) between 3 months and baseline in CD4 200 – 499 cells/mm<sup>3</sup> group (Table 4.34).

CD4/mm <sup>3</sup>	Parameter	Mean Rank	Sum of Ranks	Z- value	Asympt.
< 200	$AST E_1 - AST E_0$	4 50	4 50	-2 707	0.007*
< 200	$AST F_2 - AST F_1$	8.00	16.00	-2.065	0.039
	$ALP F_1 - ALP F_0$	12.00	12.00	-2.341	0.019
	$ALP F_2 - ALP F_0$	11.00	11.00	-2.412	0.016
	T Bil $F_1$ - T Bil $F_0$	8 00	8 00	-2.621	< 0.001*
	T.Bil $F_2$ - T.Bil $F_0$	2.00	2.00	-2.903	0.004*
	D.Bil $F_1$ - D.Bil $F_0$	0.00	0.00	-2.366	0.018*
	D.Bil $F_2$ - D.Bil $F_0$	4.00	4.00	-2.398	0.016*
	$T.PRT F_1 - T.PRT F_0$	6.08	36.50	-2.203	0.028
	$ALB F_1 - ALB F_0$	6.00	6.00	-2.598	0.009
	$ALB F_2 - ALB F_0$	3.50	7.00	-2.693	0.007
	UREA $F_2$ - UREA $F_0$	8.00	8.16	-2.062	0.039
	$CRT F_1 - CRT F_0$	4.00	4.00	-2.900	0.004*
	$CRT F_2 - CRT F_0$	3.00	9.00	-2.353	0.019*
200 - 499	AST $F_2 - AST F_0$	15.00	210.00	-3.327	0.001
	$AST F_2 - AST F_1$	12.54	175.50	-3.863	< 0.001
	GGT $F_2$ - GGT $F_0$	24.07	337.00	-2.038	0.042
	GGT F <sub>2</sub> - GGT F <sub>1</sub>	17.71	248.00	-2.884	0.004
	ALP $F_1$ - ALP $F_0$	14.47	217.00	-3.393	0.001
	$ALP F_2 - ALP F_0$	21.40	214.00	-3.280	0.001
	T.Bil $F_1$ - T.Bil $F_0$	14.88	119.00	-4.498	< 0.001*
	T.Bil $F_2$ - T.Bil $F_0$	16.88	13.005	-4.081	< 0.001*
	T.PRT $F_2$ - T.PRT $F_0$	19.00	380.00	-2.796	0.005
	ALB $F_2$ – ALB $F_0$	17.80	178.00	-3.277	0.001
	UREA $F_1$ - UREA $F_0$	17.62	299.50	-2.097	0.030
	UREA F <sub>2</sub> - UREA F <sub>0</sub>	17.19	309.50	-2.166	0.030
	$CRT F_1 - CRT F_0$	20.41	708.00	-2.150	0.032*
> 500	$AST F_2 - AST F_0$	16.68	233.50	-2.185	0.029
	$AST F_2 - AST F_1$	22.10	221.00	-2.169	0.030
	$GGT F_1 - GGT F_0$	23.90	119.50	-3.644	< 0.001
	GGT F <sub>2</sub> - GGT F <sub>1</sub>	18.31	146.50	-3.249	0.001
	ALP $F_2$ – ALP $F_0$	17.38	208.50	-2.533	0.011
	D.Bil $F_1 - D.Bil F_0$	12.44	112.00	-2.281	0.022
	T.PRT $F_1 - T.PRT F_0$	21.38	556.00.	-3.087	0.002
	T.PRT $F_2$ - T.PRT $F_1$	12.78	115.00	-3.707	< 0.001
	ALB F <sub>1</sub> - ALB F <sub>0</sub>	17.44	139.50	-3.353	0.001*
	$CRT F_2 - CRT F_0$	20.00	349.00	-2.331	0.020

Table 4.33: Differences in ART female haematological parameters during follow up

$$\label{eq:F0} \begin{split} F_{0\,=}\,Baseline,\,F_{1}{=}3\,\,months,\,F_{2}{=}\,6\,\,months.\,*\,Decrease\\ Wilcoxon\,rank-sum\,test,\,p{<}\,0.05p\,{<}\,0.05\,\,considered\,\,significant\,(2{-}tailed\,\,test) \end{split}$$

CD4/mm <sup>3</sup>	Parameter	Mean Rank	Sum of Ranks	Z- value	Asympt. sig.(2-tailed)
200 - 499	T.Bil F <sub>1</sub> - T.Bil F <sub>0</sub>	2.33	7.00	-1.836	0.043*
$\geq$ 500	D.Bil F <sub>1</sub> - D.Bil F <sub>0</sub>	12.44	112.00	-2.293	0.022
	D.Bil F <sub>2</sub> - D.Bil F <sub>0</sub>	13.64	150.00	-2.312	0.021
	T.PRT F <sub>2</sub> - T.PRT F <sub>1</sub>	3.50	7.99	-2.105	0.035

Table 4.34: Differences in ARV-naive female haematological parameters during Follow up

 $F_{0}$  = Baseline,  $F_{1}$  = 3 months,  $F_{2}$  = 6 months. \* Decrease

Wilcoxon rank-sum test, p< 0.05 considered significant (2-tailed test)

During follow up, significant increases in Biochemical parameters in ART male respondents were demonstrated in: ALP (Z = -2.023, p = 0.043) between 6 and 3 months in CD4 < 200 cells/mm<sup>3</sup> group. In CD4 200 - 499 cells/mm<sup>3</sup> group, the increases were demonstrated in: AST between 6 months and baseline (Z = -4.013, p < 0.001) and between 6 and 3 months (Z = -4.312, p < 0.001), GGT between 6 months and baseline (Z = -4.188, p < 0.001) and between 6 and 3 months (Z = -4.832, p < 0.001), ALP between 6 months and baseline (Z = -3.646, p < 0.001) and between 6 and 3 months (Z = -3.950, p < 0.001), T.Bil (Z = -2.058, p = 0.040) between 6 and 3 months, D.Bil between 3 months and baseline (Z = -2.195, p = 0.028) and between 6 months and baseline (Z = -3.178, p = 0.001); T.PRT between 6 months and baseline (Z = -3.500, p < 0.001) and between 6 and 3 months (Z = -2.716, p = 0.007); UREA between 6 months and baseline (Z = -4.022, p < 0.001) and between 6 and 3 months (Z = - 4.577, p < 0.001); CRT between 6 months and baseline (Z = -3.009, p = 0.00) and between 6 an3 months (Z = -3.489, p < 0.001). In CD4  $\geq$  500 cells/mm<sup>3</sup> group Significant increases were demonstrated in: AST (Z = -2.679, p < 0.001) between 6 and 3 months, GGT between 6 months and baseline (Z = -3.462 p = 0.001) and between 6 and 3 months (Z = - 3.524, p < 0.001), D.Bil (Z - 3.340, p = 0.001) between 6 months and baseline, T.PRT between 3 months and baseline (Z = - 2.404 p = 0.016), between 6 months and baseline (Z = -3.525, p < 0.001) and between 6 and 3 months (Z = =2.274, p = 0.023), UREA between 6 months and baseline (Z = - 3.806, p < 0.001), between 6 months and baseline (Z = -3.842, p < 0.001) and between 6 and 3 months (Z = - 3.842, p = < 0.001) and finally CRT (Z = - 2.435, p = 0.015) between 6 months and baseline. Significant decreases in the parameters were demonstrated in both T.Bil (Z = -2.023, p = 0.043) between 3 months and baseline in CD4 < 200 cells/mm<sup>3</sup> group and T.Bil (Z = - 2.783, p = 0.005) between 6 months and baseline in CD4 200 – 499 cells/mm<sup>3</sup> group (Table 4.35).

In ARV-naïve males significant increases in the parameters were demonstrated in: ALT between 6 months and baseline (Z = - 3.275, p = 0.001) and between 6 and 3 months (Z = - 4.097, p < 0.001) in CD4 200 – 499 cells/mm<sup>3</sup> group. In CD4  $\geq$  500 cells/mm<sup>3</sup> group significant increases were demonstrated in ALT between 3 months and baseline (Z = - 1.966, p = 0.049), between 6 months and baseline (Z = - 4.076, p < 0.001) and between 6 and 3 months (Z = - 3.856, p < 0.001). Decreases were demonstrated in ALT (Z = - 2.051, p = 0.046) between 3 months and baseline in CD4 200 – 499 cells/mm<sup>3</sup> group (Table 4.36).

CD4/mm <sup>3</sup>	Parameter	Mean Rank	Sum of Ranks	Z- value	Asympt. sig.(2-tailed)
< 200	ALP F <sub>2</sub> -ALP F <sub>1</sub>	0.00	0.00	-2.023	0.043
< 200	T.Bil F <sub>1</sub> - T.Bil F <sub>0</sub>	3.00	15.00	-2.023	0.043*
200 - 499	$AST E_2 - AST E_0$	8.00	32.00	-4.013	< 0.001
200 199	$AST F_2 - AST F_1$	7.00	28.00	-4 312	< 0.001
	$GGT F_2 - GGT F_0$	7.00	29.00	-4 188	< 0.001
	$GGT F_2 - GGT F_1$	1.50	1 50	-4 832	< 0.001
	$ALP F_2 - ALP F_0$	8 86	62.00	-3 646	< 0.001
	$ALP F_2 - ALP F_1$	8.10	40.50	-3 950	< 0.001
	T Bil $F_2$ - T Bil $F_0$	18 57	390.00	-2.783	0.005*
	T.Bil $F_2$ - T.Bil $F_1$	13.00	143.00	-2.058	0.040
	$\mathbf{D}_{\mathbf{B}}\mathbf{i}\mathbf{I}\mathbf{F}_{1} - \mathbf{D}_{\mathbf{B}}\mathbf{i}\mathbf{I}\mathbf{F}_{0}$	14.50	116.00	-2.195	0.028
	$\mathbf{D}.\mathbf{Bil} \mathbf{F}_2 - \mathbf{D}.\mathbf{Bil} \mathbf{F}_0$	13.00	78.00	-3.178	0.001
	T.PRT $F_2 - T.PRT F_0$	9.93	69.50	-3.500	< 0.001
	$T.PRT F_2 - T.PRT F_1$	9.95	109.50	-2.716	0.007
	UREA $F_2$ – UREA $F_0$	6.17	37.00	-4.022	< 0.001
	UREA $F_2$ – UREA $F_1$	4.83	14.50	-4.577	< 0.001
	$CRT F_2 - CRT F_0$	15.75	94.50	-3.009	0.003
	$CRT\;F_2-CRT\;F_1$	11.07	70.00	-3.489	< 0.001
> 500	$AST F_2 - AST F_1$	8 80	44 00	-2 679	< 0.001
≥ 500	$GGT F_2 - GGT F_2$	8.00	16.00	-3.462	0.001
	$GGT F_{2} = GGT F_{0}$	0.00	18.00	-3.402	< 0.001
	$DBilE_{2} DBilE_{2}$	12.00	12.00	3 340	0.001
	$\mathbf{D} \cdot \mathbf{D} \cdot \mathbf{D} \cdot \mathbf{D} = \mathbf{D} \cdot \mathbf{D} \cdot \mathbf{D} \cdot \mathbf{D} \cdot \mathbf{D} $	7 50	52.50	-3.340	0.001
	$\mathbf{1.FK1} \Gamma_1 = \mathbf{1.FK1} \Gamma_0$ $\mathbf{T} \mathbf{D} \mathbf{D} \mathbf{T} \mathbf{E} \qquad \mathbf{T} \mathbf{D} \mathbf{D} \mathbf{T} \mathbf{E}$	7.30	12.50	-2.404	0.010
	$1.PKI \Gamma_2 - 1.PKI \Gamma_0$ $T DDT E T DDT E$	0.00	18.00	-5.525	< 0.001
	$1.PKI F_2 - 1.PKI F_1$	9.42	56.50	-2.274	0.023
	UKEA $F_2$ – UKEA $F_0$	0.UU 5.00	0.00	-3.806	< 0.001
	UKEA $F_2 - UKEA F_1$	5.00	5.00	-3.842	< 0.001
	$CRT F_2 - CRT F_0$	7.36	51.50	-2.4.35	0.015

Table 4.35: Differences in ART male haematological parameters during follow up

 $F_0$  = Baseline,  $F_1$ =3 months,  $F_2$ = 6 months. \* Decrease

Wilcoxon rank-sum test, p< 0.05considered significant (2-tailed test)

 Table 4.36:
 Differences in ARV-naive male haematological parameters during follow up

$CD4/mm^3$	Donomotor	Mean	Sum of	7 voluo	Asympt. sig.(2-
CD4/11111	rarameter	Rank	Ranks	L- value	tailed)
200 - 499	$\begin{array}{c} ALT \ F_1 \ \text{-} \ ALT \ F_0 \\ ALT \ F_2 \ \text{-} \ ALT \ F_0 \end{array}$	11.96 9.00	143.50 81.00	-2.051 -3.275	0.046* 0.001
	ALT F2 - ALT F1	11.17.	33.50	-4.097	< 0.001
≥ 500	$\begin{array}{l} ALT \ F_1 \ \text{-} \ ALT \ F_0 \\ ALT \ F_2 \ \text{-} \ ALT \ F_0 \end{array}$	9.83 1.00	69.00 1.00	-1.966 -4.076	0.049 < 0.001
	ALT F <sub>2</sub> - ALT F <sub>1</sub>	8.00	8.00	-3.850	< 0.001

 $F_0$  = Baseline,  $F_1$ =3 months,  $F_2$ = 6 months. \* Decrease.

Wilcoxon rank-sum test, p< 0.05 considered significant (2-tailed test)

### 4.3.3 Levels in antiretroviral treated and untreated respondents

Among the females significant increases in Biochemical parameters between ART and ARV-naïve respondents were demonstrated in: D.Bil (Z= -2.287, p = 0.022) at baseline in CD4 < 200 cells/mm<sup>3</sup> group, D.Bil (Z = -2.428, p = 0.015) at baseline in CD4 200 - 499 cells/mm<sup>3</sup> group and ALP at baseline (Z = -2.252, p = 0.024) and at 3 months (-2.264, p = 0.024) in CD4  $\geq$  500 cells/mm<sup>3</sup> group; while decreases was demonstrated in CRT (Z = -2.208, p = 0.027) in CD4 < 200 cells/mm<sup>3</sup> group (Table 4.37).

In male respondents there was significant increase between ART and ARV-naïve respondents in ALB (Z = - 2.050, p = 0.040) and a decrease in ALP (Z = --2.115, p = 0.034) at baselines in CD4< 200 cells/mm<sup>3</sup> group (Table 4.38).

 Table 4.37: Differences in Biochemical parameters between ART and ARV- naive females.

CD4		Mean	Rank	Sum of Ranks		7	Agromatotio
cells/mm <sup>3</sup>	Parameter	ART	ARV- naive	ART	ARV- naive	Z- value	Si.(2-tailed)
< 200	D.Bil F <sub>0</sub>	6.67	12.50	80.00	25.00	-2.287	0.022
	CRT F <sub>0</sub>	9.00	1.50	117.00	3.00	-2.208	0.027*
200 - 499	D.Bil F <sub>0</sub>	22.87	34.82	892.00	383.00	-2.428	0.015
$\geq$ 500	ALP F <sub>0</sub>	25.24	38.28	1140.50	344.50	-2.252	0.024
	ALP F <sub>1</sub>	25.33	38.33	1140.00	345.00	-2.264	0.024

 $F_{0=}$ Baseline,  $F_{1=3}$  month.. \* Decrease. Wilcoxon rank-sum test, p< 0.05 considered significant (2-tailed test)

CD4		Mean l	Rank	Sum of H	Ranks	7	Agromatotio
cells/mm <sup>3</sup>	Parameter	ART	ARV- naive	ART	ARV- naive	z- value	Si.(2-tailed)
< 200	ALP F <sub>0</sub>	17.90	3.00	555.00	6.00	-2.115	0.034*
< 200	ALB F <sub>0</sub>	16.13	30.50	500.00	61.00	2.050	0.040

### Table 4.38: Differences in Biochemical parameters between ART and ARV- naïve males

 $F_0$  = Baseline. \* Decrease. Wilcoxon rank-sum test, p< 0.05 is considered significant (2-tailed test)

### 4.3.4 Levels within CD4 groups

Among the females, significant differences in Biochemical parameters within the CD4 groups were demonstrated in: AST ( $X^2 = 13.732$ , p = 0.001), T.Bil. ( $X^2 = 20.198$ , p < 0.001), D.Bil ( $X^2 = 6.696$ , p = 0.035), ALB ( $X^2 = 13.726$ , p = 0.001) CRT ( $X^2 = 0.457$ , p = 0.040) at baseline and ALT ( $X^2 = 8.894$ , p = 0.012) at 6 months in ART respondents (Table 4.39). In ARV-naïve females significant differences were demonstrated in: T.Bil ( $X^2 = 6.685$ , p = 0.035) and D.Bil ( $X^2 = 9.570$ , p = 0.008) at baseline (Table 4.40).

Table 4.39: Within group changes in Biochemical parameters in ART females

Parameter	Chi-square (X <sup>2</sup> )	Df	Asymptotic sig. (2-tailed)
AST F <sub>0</sub>	13.732	2	0.001
ALT F <sub>2</sub>	8.894	2	0.012
T.Bil F <sub>0</sub>	20.198	2	< 0.001
D.Bil F <sub>0</sub>	6.696	2	0.035
ALB F <sub>0</sub>	13.726	2	0.001
CRT F <sub>0</sub>	0.457	2	0.040

Kruskal –Wallis test, p < 0.05 is significant  $F_0$  = Baseline,  $F_2$  = 6 months

Table 4.40: Within group changes in Biochemical parameters in ARV-naive females

Parameter	Chi-square (X <sup>2</sup> )	Df	Asymptotic sig. (2-tailed)
T.Bil F <sub>0</sub>	6.685	2	0.035
D.Bil F <sub>0</sub>	9.570	2	0.008

Kruskal –Wallis test, p < 0.05 is significant  $F_{0=}$  Baseline

Among the males significant differences in Biochemical parameters were demonstrated in: ALT( $X^2 = 7.984$ , p = 0.018) at 6 months, ALP ( $X^2 = 9.143$ , p = 0.010) at 3 months and ALB ( $X^2 = 6.466$ , p = 0.039) at baseline in ART respondents (Table 4.41).

Table 4.41: Within group changes in Biochemical parameters in ART males

Parameter	Chi-square (X <sup>2</sup> )	Df	Asymptotic sig. (2-tailed)
ALT F <sub>2</sub>	7.984	2	0.018
ALP F <sub>1</sub>	9.143	2	0.010
ALB F <sub>0</sub>	6.466	2	0.039

 $F_0$  = Baseline,  $F_1$ =3 months,  $F_2$ = 6 months. Kruskal – Wallis test, p < 0.05 is significant

## 4.4 Effects of HIV infection in male and female subjects on the levels of Epo, TNF-α, IL-6 and CRP parameters

### 4.4.1 Baseline levels

Among females significant increases in median values between referents and ART respondents were demonstrated in: Epo (Z = -4.955, p< 0.001) and TNF- $\alpha$  (Z = -5.043, p < 0.001) in CD4 < 200 cells/mm<sup>3</sup> group, Epo (Z = -8.558, p< 0.001) and TNF- $\alpha$  (Z = -7.468, p < 0.001) in CD4 200-499 cells/mm<sup>3</sup> group, Epo (Z = -7.915, p< 0.001) and TNF- $\alpha$  (Z = -6.596, p< 0.001) in CD4  $\geq$  500 cells/mm<sup>3</sup> group. Decreases were demonstrated in: IL-6 (Z = -2.569, p = 0.010), CRP (Z = -3.269, p = 0.001) in CD4 200 - 499 cells/mm<sup>3</sup> group and CRP (Z = -3.278, p < 0.001) in CD4  $\geq$  500 cells/mm<sup>3</sup> group (Table4.42)

In ARV-naïve respondents significant increases were demonstrated in each of: TNF- $\alpha$  (Z= -2.552, p = 0.011) in CD4 < 200 cells/mm<sup>3</sup> group, Epo (Z = -4.318, p< 0.001) and TNF- $\alpha$ 

$$(Z = -4.624, p < 0.001)$$
 in CD4 200 – 499 group, Epo  $(Z = -4.791, p < 0.001)$  and TNF- $\alpha$   
 $(Z = -3.672, p < 0.001)$  in CD4  $\geq$  500 cells/mm<sup>3</sup> group (Table 4.43)

Table 4.42: Differences between referents and ART female baseline cytokine values

CD4/mm <sup>3</sup>	Doromotor	Mear	n Rank	Sum of	Ranks	7 voluo	Asymptotic
	rarameter	$\mathbf{F}_{\mathbf{N}}$	F <sub>0</sub>	$\mathbf{F}_{\mathbf{N}}$	$\mathbf{F}_{0}$	L-value	Sig. 2-tailed
< 200	Epo	55.51	106.62	5995.00	1386.00	-4.955	< 0.001
	TNF-α	55.89	103.42	6036.50	1344.50	-5.043	< 0.001
200 - 499	Epo	57.21	124.49	6179.00	5602.00	-8.558	< 0.001
	TNF-α	60.47	116.67	6534.00	5250.00	-7.468	< 0.001
	IL-6	82.37	64.12	8895.50	2885.50	-2.569	0.010*
	CRP	84.53	58.93	9129.00	2652.00	-3.269	0.001*
≥ 500	Epo	52.24	120.41	6182.00	4696.00	-7.943	< 0.001
	TNF-α	60.82	110.50	6568.50	4309.50	-6.596	< 0.001
	CRP	80.87	54.99	8733.50	2144.50	-3.278	< 0.001*

Wilcoxon rank-sum test, p< 0.05, considered significant (2-tailed test). \* Decrease  $F_N$  = Referents,  $F_0$  = Baseline.

•/	<b>Table 4.43:</b>	Differences	between	referents	s and AF	<b>RV-naive</b>	female	baseline	cytokine
----	--------------------	-------------	---------	-----------	----------	-----------------	--------	----------	----------

CD4/mm <sup>3</sup>	Donomotor	Mear	n Rank	Sum of	Ranks	7 voluo	Asymptotic
	rarameter	$\mathbf{F}_{\mathbf{N}}$	F <sub>0</sub>	$\mathbf{F}_{\mathbf{N}}$	$\mathbf{F}_{0}$	Z-value	Sig. 2-tailed
< 200	TNF-α	54.56	106.00	5893.00	212.00	-2.552	0.011
200 - 499	Epo	55.09	105.89	5950.00	953.00	-4.318	< 0.001
	TNF-α	55.21	104.50	5962.50	940.50	-4.624	< 0.001
≥ 500	Epo	47.81	89.25	4494.00	357.00	-4.791	< 0.001
	TNF-α	48.14	81.50	4525.00	326.00	-3.672	< 0.001

Wilcoxon rank-sum test, p < 0.05 considered significant (2-tailed test).  $F_N = Referents, F_0 = Baseline. * Decrease$ 

Significant increases in proinflammatory cytokine parameters between referents and HIV infected ART male respondents were demonstrated in: Epo (Z = -2.624, p< 0.001) in CD4 < 200 cells/mm<sup>3</sup> group, Epo (Z = -6.418, p< 0.001) and TNF- $\alpha$  (Z = -4.713, p<0.001) in CD4 200 – 499 cells/mm<sup>3</sup> group, Epo (Z = -5.597, p< 0.001), TNF- $\alpha$  (Z = -3.940 p< 0.001)

and IL-6 (Z = -2.752, p = 0.005) in CD4  $\geq$  500 cells/mm<sup>3</sup> group; while a decreases was demonstrated in IL-6 (Z= -2.549, p = 0.011) in CD4 200 – 499 cells/mm<sup>3</sup> group (Table 4.44). In ARV – naïve males the increase were demonstrated in Epo (Z = -2.859, p = 0.004) and TNF- $\alpha$  (Z = -2.348, p = 0.019) in CD4  $\geq$  500 cells/mm<sup>3</sup> group (Table 4.45)

CD4/mm <sup>3</sup>	Doromotor	Mean	Rank	Sum of	Ranks	Z-	Asymptotic
< 200	I al allietel	$\mathbf{F}_{\mathbf{N}}$	$\mathbf{F}_{0}$	$\mathbf{F}_{\mathbf{N}}$	F <sub>0</sub>	value	Sig. (2-tailed)
< 200	Epo	48.26	82.80	4536.00	414.00	-2.624	< 0.001
200 - 499	Epo	51.06	99.19	4800.00	3075.00	-6.418	< 0.001
	TNF-α	54.32	89.32	5106.00	2760.00	-4.713	< 0.001
	IL-6	67.55	49.21	6349.50	1525.50	-2.549	0.011*
≥ 500	Epo	50.05	94.59	4705.00	2081.00	-5.597	< 0.001
	TNF-α	52.64	83.50	4948.00	1838.00	-3.940	< 0.001
	IL-6	62.47	41.55	5872.00	914.00	-2.762	0.006

Table 4.44: Differences be	etween referents a	and ART ma	le baseline c	ytokine values
----------------------------	--------------------	------------	---------------	----------------

Wilcoxon rank-sum test, p < 0.05, considered significant (2-tailed test).  $F_N = Referents, F_0 = Baseline. * Decrease$ 

### Table 4.45: Differences between referents and ARV male baseline cytokine values

$CD4/mm^3$	Domomotor	Mean	Rank	Sum of	Ranks	7 voluo	Asymptotic
CD4/IIIII	1 al allieter	$\mathbf{F}_{\mathbf{N}}$	F <sub>0</sub>	$\mathbf{F}_{\mathbf{N}}$	$\mathbf{F}_{0}$	<b>Z</b> -value	Sig. 2-tailed
≥ <b>500</b>	Epo	47.81	89.25	4494.00	357.00	-2.859	0.004
	TNF-α	48.14	81.50	4525.00	326.00	-2.348	0.019

Wilcoxon rank-sum test, p< 0.05, considered significant (2-tailed test).  $F_N = Referents, F_0 = Baseline.$ 

### **4.4.2 Longitudinal levels**

During the 6 months of the study significant increase in proinflammatory cytokine in ART females were demonstrated in TNF- $\alpha$  between 3 months and baseline (Z = -2.341, p = 0.019) in CD4 < 200 cells/mm<sup>3</sup> group and between 3 months and baseline (Z = -2.715, p = 0.001) in CD4 200 - 499 cells/mm<sup>3</sup> group (Table 4.46). In ARV-naïve females

significant increases were demonstrated in CRP (Z = -2.701, p= 0.007) between 6 months and baseline in CD4  $\geq$  500 cells/mm<sup>3</sup> group (Table 4.47).

CD4/mm <sup>3</sup>	Parameter	Mean Rank	Sum of Ranks	Z- value	Asympt. sig.(2-tailed)
< 200	TNF- $\alpha$ F <sub>1</sub> - TNF- $\alpha$ F <sub>0</sub>	3.00	12.00	-2.341	0.019
200 - 499	TNF- $\alpha$ F <sub>1</sub> - TNF- $\alpha$ F <sub>0</sub>	21.31	277.00	-2.715	0.007

Table 4.46: Differences in ART female cytokine parameter
--

 $F_0$  = Baseline,  $F_1$ =3 months, F2= 6 months. Wilcoxon rank-sum test , p< 0.05, considered significant 2-tailed test)

Table 4.47: Differences in ARV-naive female cytokine parameters du	uring t	follow u	p
--	---------	----------	---

CD4/mm <sup>3</sup>	Parameter	Mean Rank	Sum of Ranks	Z- value	Asympt. sig.(2-tailed)
200 - 499	TNF- $\alpha$ F <sub>1</sub> - TNF- $\alpha$ F <sub>0</sub>	0.00	0.00	-2.668	0.008?
	TNF- $\alpha$ F <sub>2</sub> - TNF- $\alpha$ F <sub>1</sub>	0.00	0.00	-2.666	0.008?
	CRP F <sub>1</sub> - CRP F <sub>0</sub>	3.00	3.00	-2.100	0.036?
	CRP F <sub>2</sub> - CRP F <sub>0</sub>	2.00	2.00	-2.245	0.025?
≥ 500	$CRP \ F_2 - CRP \ F_0$	2.50	10.00	-2.701	0.007

 $F_0$  = Baseline,  $F_1$ =3 months,  $F_2$ = 6 months. ? Disregarded due to similar rank values Wilcoxon rank-sum test, p< 0.05, considered significant (2-tailed test)

In ART males significant increase in proinflammatory cytokine during follow up were demonstrated in: TNF- $\alpha$  (Z = -3.488, p< 0.001) between 3 months and baseline and in IL-6 (Z = -2.327, p = 0.020) between 6 and 3 months in CD4 200 – 499 cells/mm<sup>3</sup> group; TNF- $\alpha$  (Z = -3.459, p = 0.001) in CD4 ≥500 cells/mm<sup>3</sup> group. Conversely significant differences were demonstrated in: TNF- $\alpha$  (Z = -2.470, p = 0.014) between 6 and 3 months and IL-6 (Z = -2.058, p = 0.040) between 3 months and baseline in CD4 200 – 499 cells/mm<sup>3</sup> group and TNF- $\alpha$  (Z = -2.856, p = 0.001) between 6 and 3 months in CD4 ≥500 cells/mm<sup>3</sup> group (Table 4.48)

CD4/mm <sup>3</sup>	Parameter	Mean Rank	Sum of Ranks	Z- value	Asympt. sig.(2-tailed)
200 - 499	TNF-a $F_1$ - TNF-a $F_0$	11.67	426.00	-3.488	< 0.001
	TNF-a $F_2$ - TNF-a $F_1$	13.56	122.00	-2.470	0.014*
	IL-6 F <sub>1</sub> - IL-6 F <sub>0</sub>	11.14	7800	-2.058	0.040*
	IL-6 F <sub>2</sub> - IL-6 F <sub>1</sub>	8.79	61.50	-2.327	0.020
$\geq$ 500	TNF- $\alpha$ F <sub>1</sub> -TNF- $\alpha$ F <sub>0</sub>	5.33	16.00	-3.459	0.001
	TNF- $\alpha$ F <sub>2</sub> - TNF- $\alpha$ F <sub>1</sub>	7.13	28.50	-2.856	0.004*

 Table 4.48: Differences in ART male cytokine parameters during follow up

 $F_0$  = Baseline,  $F_1$ =3 months,  $F_2$ = 6 months. \* Decrease Wilcoxon rank-sum test, p< 0.05, considered significant (2-tailed test)

### 4.4.3 Levels in antiretroviral treated and untreated respondents

Significant increase in proinflammatory cytokine median values between ART and ARVnaïve females was demonstrated in TNF- $\alpha$  (Z = -2.418, p = 0.016) at 6 months and decrease in CRP (Z = -2.535, p = 0.011) at baseline in CD4 200 – 499 cells/mm<sup>3</sup> group (Table 4.49). In males, the increase was demonstrated in Epo (Z = -2.263, p = 0.024) at 3 months and the decrease in TNF- $\alpha$  (Z = -2.151, p = 0.031) at baseline in CD4 200 – 499 cells/mm3 group (Table 4.50).

		Mean	Rank	Sum of R	lanks	7-	Asympt sig
CD4 counts	Parameter	ART	ARV- naive	ART	ARV- naive	value	(2-tailed)
200 400	TNF- $\alpha$ F <sub>2</sub>	25.19	39.06	1133.50	351.50	-2.418	0.016
200-499	CRP F <sub>0</sub>	29.89	15.56	1345.00	140.00	-2.535	0.011*

Table 4.49: Differences between ART and ARV-naïve females in cytokines

 $F_{0=}$ Baseline,  $F_{1}$ =3 months. \* Decrease. Wilcoxon rank-sum test, p< 0.05, considered significant (2-tailed test)

CD4 counts	Parameter	Mean Rank		Sum of Ranks		7.	Asympt.	
		ART	ARV- naive	ART	ARV- naive	value	sig. (2- tailed)	- F
200-499	Epo F <sub>1</sub>	16.03	32.00	497.00	64.00	-2.263	0.024	$\mathbf{F}_{0} =$
	TNF- $\alpha$ F <sub>0</sub>	17.92	2.75	555.50	5.50	-2.151	0.031*	

Table 4.50: Differences between ART and ARV-naïve males in cytokines

Baseline, F<sub>1</sub>=3 months. \* Decrease. Wilcoxon rank-sum test, p< 0.05, considered significant (2-tailed test)

#### **CHAPTER FIVE**

### DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

### 5.1 Discussion

Human immunodeficiency virus (HIV) - associated anaemia is the commonest cytopenia associated with the disease progression and poor clinical outcomes. Early diagnosis and proper classification of the anaemia prospectively will be vital in instituting the appropriate managerial interventions for the anaemia and HIV disease. Accurate interpretation of clinical laboratory results requires reliable gender- based reference ranges preferably derived from referent cohorts with similar social-demographic characteristics as the test respondents (Zeh *et al.*, 2011).

In this study, the observed significantly higher red blood cells (RBCs), haemoglobin (HB) and eosinophils (E) mean values and lower CD4+ cells, white blood cells (WBC), lymphocytes (L), neutrophils (N) and platelets (P) mean values in referent male than in referent females have been reported in other studies (Ngowi *et al.*, 2008; Miri-Dashe *et al.*, 2014; Addal-Mensah *et al.*, 2019). This has been attributed to the direct effect of sex hormones, androgens which are associated with rises in erythropoietin levels which consequently increase bone marrow activity and enhance entry of iron into erythrocytes (Murphy, 2014); testosterone hormone which enhances erythropoiesis by promoting iron metabolism and increasing iron bioavailability (Beggs *et al.*, 2014).and to menstrual blood flow in women (Kibaya *et al.*, 2008; Zeh *et al.*, 2011).
The demonstrated significantly higher mean values in males than females in: AST, ALT, ALP, ALB and CRT has been reported from other studies (Saathoff *et al.*, 2008; Karita *et al.*, 2009; Waithaka *et al.* 2009; Timbre *et al.*, 2014). However, gender-based insignificant differences in gamma glutamyl transferase (GGT) observed and females demonstrating significantly higher protein mean values than males have not been reported in the other studies.

Significantly higher median values of Epo, TNF-  $-\alpha$  and IL-6 and lower CRP in male than in females were demonstrated but could not be compared with results from any study conducted among Africans as none was found.

Anaemia has been reported as the main haematological complication in HIV infection (Semba and Gray, 2001; Redig and Berliner, 2013) and various types have been described: normocytic normochromic (63.88%), microcytic hypochromic (19.44%) and macrocytic normochromic (34.62%) in antiviral-treated HIV (HIV-ART) patients (Mathews *et al.* 2013). The distribution of these anaemias among male and female genders and across the CD4 counts was not described. In the current study, microcytic hypochromic anaemia of up to: 15.4% in ART females, 50% in antiretroviral (ARV) - naïve females and 50% in ART males distributed across the different CD4 clusters accompanied with rouleaux formation were demonstrated. High levels of anaemia in female respondents could partly be attributed to increased iron needs in child-bearing (Hallbergl, 1988; Earl and Woteki, 1993; Ansari *et al.*, 2009; Mawani *et al.*, 2016). Longitudinal changes showed significant increases in RBC and HB levels in ART within CD4 < 200 cells group and above ARV-naive females across CD4 groups. This was possibly a sign of ameliorated anaemia in the

former due to ARV therapy. Causes of microcytic hypochromic cells include: iron deficiency, thalassemia and chronic illness and chronic blood loss (Ford, 2013). Thalassemia and chronic blood loss were ruled out from blood film examination by excluded haemolytic features such as polychromatic cells, schistocytes or normoblasts (Naeim *et.al.* 2018). Chronic illness could be attributed to HIV infection and/or co-infections. In HIV infection dyserythropoiesis including poor iron metabolism has been reported (Kupka *et al.*, 2007). Iron deficiency could not be ruled out because iron assays were not carried out. It has been reported that rouleaux formation, anaemia, hypovolemia, acute - phase proteins (like fibrinogen) and low albumin levels are associated (Fabre, 1987; Huang, 1987). In this study anaemia was demonstrated hypovolemia was excluded clinically by ruling out typical signs such as vomiting, diarrhea, decreased intake and decreased pulse rates (Sinert and Spektor, 2005). Low ALB levels were ruled out but acute-phase proteins were not assayed.

Conversely, macrocytic anaemia presents in two forms: megaloblastic and nonmegaloblastic. The former presents with oval macrocytes and the later with round macrocytes. Megaloblastic anaemia is caused by vitamin  $B_{12}$  or folate deficiencies, or DNA-replication poisons (Kaferle and Strzoda, 2009); while non-megaloblastic which emanate from membrane excesses due to excess lipid deposition on the cell membrane (Veda, 2012), is associated with: alcoholism, liver disease, chronic renal failure, aplastic anaemia, myelodysplasia, hypothyroidism, autoimmune disorders and adverse effects of some drugs used for treatment (Kaferle and Strzoda, 2009; Veda, 2012). Indeed macrocytosis is frequent in patients receiving zidovudine therapy (Richman *et al.*, 1987; Oliveira *et al.*, 2011). In this study non-megaloblastic anaemia of up to: 28.2%, 50% in ART and ARV-naïve females respectively and 26% in both ART and ARV-naïve males accompanied with target cells was demonstrated. Target cells are associated with haemoglobinopathies and liver diseases (Kaewketthong, Bunyaratvej and Barusrux, 1992). Clinical history ruled out any features of hypothyroidism such as motor activity, constipation, cold intolerance, menorrhagia, stiff muscles, sleep apnea, dry skin, weight gain, snoring, and hoarse voice (Eduardo and Davd, 1997; El-Shafie, 2003). Myelodysplasia would have been considered if neutropenia, thrombocytopenia, anaemia, dysplastic changes like basophilic stippling and Howell-Jolly bodies were demonstrated during blood film evaluation (Foran and Shammo, 2012). Antiretroviral drugs, alcoholism, transient liver and/or kidney disorders are most likely responsible for the observed macrocytosis singly or in combination.

Pancytopenia has been described as a common feature in HIV infection (Edberg *et al.*, 2015; Vishnu and Aboulafia, 2015), arising from mechanisms such as immune mediated destruction of platelets and bone marrow suppression by HIV infection resulting in decreased production of blood cells (Addis *et al.*, 2014). From the current study, however, the frequency of the cytopenias demonstrated were much lower than those reported from other studies. Examples: in ART females across the three CD4 groups neutropenia of 2.2 - 10.3% versus 70% and thrombocytopenia of 2.2 - 5.1% versus 30-40% (Santis *et al.* 2011). The main leucocyte cytopenia demonstrated was lymphopenia of up to 33% in ART females and 50% in ARV males with the highest levels in lowest CD4 count (< 200 cells and 200 – 499 cells/mm<sup>3</sup> groups in females and males respectively). The pathology was

significantly higher in ART than ARV-naïve females. The role of ARV therapy in this would be best elucidated if similar sample sizes for males and females were studied. Various levels of lymphopenia in HIV infection have been reported: 65.2 % from 250 ARV- naïve patients (Parinitha and Kulkarni, 2012), 28% from 204 ART patients (Rahman *et al.*, 2014) and 23% from ARV – naïve patients (Devi *et al.*, 2016). In these studies, the majority of cases were seen in CD4 < 200 cells/mm<sup>3</sup> group, a trend comparable to the findings of this study. The observed trend in lymphopenia levels supports the recommendation of using absolute lymphocyte counts in monitoring HIV treatment in resource – limited setting (Ryst *et al.*, 1998; Gelaw *et al.*, 2013). Lymphopenia arises from depletion of lymphocytes through antibody-dependent cytotoxicity mechanisms (Mikal, 1993). Save for WBC in ARV – naïve females, the effect of CD4 counts on all the other blood cell counts was sporadic and resolved with time.

In this study nevertheless, across the CD4 groups: neutrophilia of up to: 15% in ART females and 25% in ART males, eosinophilia of up to11.1% in ART females, thrombocytosis of up to11.1% in ART females and up to 25% in CD4  $\geq$  500 cells/mm<sup>3</sup> group in the male respondents was demonstrated. Eosinophilia levels were significantly higher in ART than in ARV-naïve females in CD4 < 200 cells/mm<sup>3</sup> group. During the study period, the levels of neutrophilia and thrombocytosis significantly increased in both ART females and males. These observations suggest that either ARVs, are ineffective in arresting the pathological increases in the blood cells or stimulate the processes altogether. In deed ARVs have been reported to cause acute inflammatory responses in some occasion when there is erratic immune restoration (Wilson and Sereti.2013). Neutrophilia and

eosinophilia are associated with chronic and acute bacterial or viral infections and inflammatory states (Rao *et al.*, 2018); while thrombocytosis in HIV has been reported to arise from compensatory thrombopoietin production (Kazama *et al.*, 2011). Thrombopoietin assay was not done in the current study

Granulocyte hypersegmentation has earlier been associated with megaloblastic anaemias arising from vitamin  $B_{12}$  or folate deficiencies, or DNA-replication poisons (Kaferle and Strzoda, 2009), but recent reports have associated it with iron deficiency anaemia (Westerman *et al.*, 1999; Sipahi *et al.*, 2002; Düzgün *et al.*, 2005; Özcan, 2011). Coupled with the presence of microcytic hypochromic cells observed, this study supports the later reports although iron assays would be helpful.

In Biochemistry both ART and ARV- naive females and ART males demonstrated significant increases in: ALT, AST and GGT in all CD4 groups; ALB and ALP in ART and ARV-naïve females in CD4 less than 500 cells/mm<sup>3</sup> groups<sup>-</sup> These increases were demonstrated throughout the study period. The increase in ALP was higher in ART than ARV-naïve females. Increases in AST and ALT have been reported in myocardial infarction, acute liver cell damage, viral hepatitis and carbon tetrachloride poisoning (Netto *et al.*, 2009; Dusingize *et al.*, 2015). Moreover, increases in both GGT and ALP have been reported in biliary disease (Lum and Gambino, 1972; Hyder *et al.*, 2013). With the exclusion of respondents with known liver disorders during recruitment, liver damage would only be transient during the study period and cardiac disorders would only be ruled out by evaluation of cardiac enzymes such as troponin T and troponin I.

Significant decreases in T.PRT in all CD4 groups of ART and in > 200 cells/mm<sup>3</sup> groups and in CD4 < 500 cells/mm<sup>3</sup> groups in ARV-naïve females. Observed increases in ALB levels indicate increased albumin: globulin ratio below the normal of 60%:40% (Levin and Egorihima, 2013). Antibodies fall in globulin fraction of total protein and thus decrease in globulins may be indicative of low antibody levels and this can be confirmed by determining antibody titers. Although T.Bil and D.Bil parameters were generally decreased to the study in all respondents, this was not considered to be of any clinical significant. Discrepancies in the increases and decreases between female and male respondents may require further studies using equal sample sizes for the groups to amplify any gender-based influences. There was no established effect of CD4 counts on the Biochemical parameters throughout the study.

Significant increases in Epo and TNF together with decreases in CRP and IL- 6 levels were demonstrated during recruitment but only TNF- $\alpha$  remained progressively increased in the entire study period in ART females. Increases in Epo and TNF- $\alpha$  in ARV-naïve females stabilized after 3 months of the study and CRP increased thereafter to the end of the study. On the other hand ART males demonstrated significant increase in 1L-6 levels in  $\geq$  500 cells/mm<sup>3</sup> group and a decrease in 200 – 499 cells/mm<sup>3</sup> group. These desynchronized changes in levels of the parameters between female and male respondents can only be clearly understood if equal sample sizes of the genders are evaluated over a longer study period. ART females demonstrated higher TNF- $\alpha$  and lower CRP levels than ARV – naïve females; while in males Epo levels were significantly higher and TNF- $\alpha$ lower in ART than in ARV groups. These observations are suggestive of TNF- $\alpha$  as the main proinflammatory cytokines in HIV infection; also the levels are in agreement with the reports by Aukrust *et al.* (1999) and Khanna *et al.* (2017) that TNF- $\alpha$  levels increase in HIV infection.

The demonstrated increases in Epo are in keeping with the other reports that Epo levels increase in HIV infection (Amanzada *et al.*, 2014; Beverborg *et al.*, 2015). Notwithstanding, presence of high levels of anaemia observed among the study respondents contradicts reports that attribute HIV-associated anaemia to Epo deficiency as a result of destruction by circulating autoantibodies arising from molecular mimicry between Epo and HIV-1 p17 protein (Tsiakalos *et al.*, 2011). The current study findings suggest that the most likely cause of anaemia could be hyposensitivity of Epo or Epo resistance by erythroblasts as reported by Agarwal *et al.*, (2008). Furthermore, high levels of TNF- $\alpha$  cause anaemia in HIV by suppressing bone marrow (Ellaurie and Rubinstein, 1995; Sade-Feldman, *et al.*, 2013; Kalyani and Jamil, 2015) and promoting apoptosis of erythroid precursor cells (Kreuzer and Rockstroh, 1997; Morceau *et al.*, 2009).

Reports available indicate that IL-6 levels increase in HIV infection (,Henrik *et al.*, 1996), the observed decreases in the current study is not clear but the role of high levels of high-affinity autoantibodies to IL-6 reported in the serum of some blood donors (Ridker *et al.*, 2000; Fosgerau *et al.*, 2009) need to be explored. Increases in CRP levels in ARV-naïve respondents above ART respondents may be suggestive of remission following ART.and this supports the use of CRP levels in monitoring disease progress or the effectiveness of treatment (Brian and Olshaker, 1999; Jain *et al.*, 2011).

#### **5.2 Conclusions**

From the study findings, the following conclusions were made:

# i. On gender-specific 95% reference intervals for haematological and Biochemical parameters, and Epo, II-6, TNF-α and CRP values

- a) Significantly higher RBC, HB, E and lower WBC, N, L and P mean values in males than in females were demonstrated in agreement with other study reports.
- b) Significantly higher AST, ALT, ALP, ALB and CRT mean values in male than in female subjects as reported elsewhere were demonstrated.
- c) No significant gender-based differences in GGT mean values although review of other reports indicates significantly higher values in males.
- d) The mean values of total protein obtained in this study were significantly higher in females than in males; other reports indicate that they are higher.in males.
- e) Significantly higher CRP, TNF and IL-6 and lower IL-6 median values in males than in females were demonstrated.

### ii. On the effects of HIV infection in female and male subjects on the blood cell morphology and haematological parameters

- a) Microcytic hypochromic anaemia of up to 15.4%, 50% in ART, ARV-naive females respectively and 50% in ART males accompanied by rouleaux formation across the CD4 groups was demonstrated.
  - b) Demonstrated significant increase in RBC and HB mean values in ART above ARV-naïve females in CD4 < 200 cells group was attributed to possibly the therapeutic effect of ARVs.

- c) Microcytic hypochromic anaemia was suspect to arise from chronic illness and iron deficiency; iron assays would give clear diagnosis.
- d) High levels of anaemia in females was thought to arise from the increased iron needs in child-bearing age.
- e) Rouleaux formation was thought to be due to anemia and presence of acute phase proteins and evaluation of the later would be confirmatory.
- f) Non-megaloblastic anaemia of up to 28.2 %, 50% in ART and ARV-naive females and 25% in both ART and ARV-naïve males accompanied with target cells was demonstrated.
- g) The cause of the anaemia was thought to be ARVs, alcoholism, transient liver and kidney disorders singly or in multiples.
- h) Target cells were thought to arise from liver disorders.
- i) Neutophilia of up to 15%, 25% in ART and ARV-naïve females respectively and 25% in both ART and ARV-naïve males; eosinophilia of up to 11.1% in ART females; thrombocytosis of up to 11.1% in ART females and up to 25% in male respondents in CD4 ≥ 500 cells/mm<sup>3</sup> group; granulocyte hypersegmentation were demonstrated.
- j) During follow up, neutrophilia and thrombocytosis significantly increased in both ART females and males.
- k) Neutrophilia and eosinophilia were thought to be associated with bacterial or viral infections and inflammatory states; hypersegmentation was suspected to be associated with iron deficiency.
- 1) Thrombocytosis was thought to arise from compensatory thrombopoietin

production, but thrombopoietin assay would be confirmatory.

- m) The main demonstrated cytopenia was lymphopenia of up to 33% in ART females and 50% in ARV males and the levels decreased with increases in CD4 counts.
- n) The findings of the current study support other reports in which the use of lymphocyte absolute counts in place of CD4+ T lymphocyte counts in resourcelimited setting is recommended.
- o) Thrombocytopenia develops through antibody-dependent cytotoxicity mechanisms.

## iii. On effects of HIV infection in female and male subjects on the levels of biochemical parameters

- a) Throughout the study period, significant increases in AST, ALT, and GGT were demonstrated in both ART and ARV-naïve females in all CD4 groups; while increases in ALB and ALP were demonstrated in ARV-naïve females in CD4 < 500 cells/mm<sup>3</sup> groups.
- b) Increases in AST and ALT was thought to be due to transient liver disorder and/ or myocardial infection; evaluation of cardiac enzymes such as troponin T and I would be helpful.
- c) Increases in GGT and ALP was thought to be associated with biliary disease. Significant decreases in T.PRT in ART and ARV-naïve females, with the observed increases in ALB levels indicate increased albumin: globulin ratio and may be indicative of low antibody levels; determination of immunoglobin titers would be confirmatory.

d) The demonstrated increases and decreases in the Biochemical parameters were only in females; any significant gender-based differences can be elucidated if studies are done using equal sample sizes for each gender and for longer period.

## iv. On effects of HIV infection in female and male subjects on the levels of Epo, TNF-α, IL-6 and CRP

- a) Demonstration of baseline increases in Epo and TNF-α and decreases in CRP and IL-6 levels but TNF-α increases persisting throughout the study period in ART females was done..
- b) In ARV-naïve females, increases in Epo and TNF- $\alpha$  stabilized at the end of the study after 3 months; while in ART males increases in IL-6 levels in CD4  $\geq$  500 and decreased in 200 499 cells/mm<sup>3</sup> groups.
- c) The gender based differences demonstrated in the levels of the cytokines could not be explained and studies involving equal numbers of each gender for longer periods would be helpful.
- d) TNF- $\alpha$  was suggested as the main proinflammatory cytokine in HIV.
- e) These observations are suggestive of TNF- $\alpha$  as the main proinflammatory cytokines in HIV infection.
- f) Demonstrated high levels of Epo in the presence of anaemia suggests that anti- Epo autoantibodies, hyposensitivity or resistance of Epo by erythroblasts was the cause of HIV- associated anaemia.
- g) High levels of TNF- $\alpha$  demonstrated may also contribute to anaemia by suppressing bone marrow and promoting apoptosis of erythroid precursors.

- h) Decreases in IL-6 levels demonstrated could be possibly due to high levels of high affinity autoantibodies to IL-6 but this requires to be investigated.
- i) Increases in CRP levels in ARV-naïve respondents above ART respondents may be suggestive of remission following ART.

#### **5.3 Recommendations**

#### **5.3.1 Recommendation from the study**

From the findings of this study, the following recommendations are made:

- Use of the established RBC, HB, WBC, N, L, E, P, ALT, AST, ALP, ALB, CRT,
   Epo, TNF-α, IL-6 and CRP ranges as gender-based reference values in Kenya.
- ii. Use of ARVs to ameliorate anaemia in HIV.
- iii. Use of iron concentrates in the management of microcytic hypochromic anaemia especially in adult females if low serum iron levels are confirmed.
- iv. Microscopic evaluation of blood films in non-alcoholic HIV patients on ARV for round macrocytes to rule out early hepatic and/or renal disorders.
- v. Use of lymphocyte absolute counts to monitor progress of HIV infection in resource-limited setting.
- vi. Incorporate AST, ALT, GGT, ALP, T.PRT and ALB in the routine management of HIV-Infection
- vii. Assay of Epo levels in HIV-associated anaemia
- viii. Determination of TNF- $\alpha$  levels in inflammatory disorders.
- ix. Assay of CRP levels in HIV patients on antiretroviral therapy.

### 5.3.2 Recommendations for future study

- i. Assay of iron levels in HIV-associated anaemia
- ii. Assay of thrombopoietin levels in HIV- Infection.
- iii. Studies on the mechanism of Epo hyposensitivity or resistance by erythroblasts in

HIV-associated anaemia.

- v. Incorporation of cardiac enzyme assays in HIV management to rule out cardiac disorders.
- vi. Monitoring the changes demonstrated in the studied parameters using larger sample sizes with equal male and female respondents for longer periods.

#### REFERENCES

- Abdelfatah, M.M. and Tuttle, B. (2017). A Rare Gastrointestinal Presentation in an HIV-Infected Patient. *Gastroenterology*.152:e7–e9
- Adewoyin, A.S. and Nwogoh, B. (2014). Peripheral Blood Film A review. Annals of Ibadan Postgraduate Medicine, 12 (2): 71–79.
- Adewumi, A.A., Titilope, A.A. and Osamuedemen, V.A. (2014). Prevalence of HIVrelated autoimmune haemolytic anaemia in Lagos, Nigeria. *Nigerian Medical Journal*, 55(1): 63–66.
- Addis, Z., Yitayew, G. and Tachebele, B. (2014). Prevalence of Some Haematological Abnormalities among HIV Patients on Their First Visit to a Tertiary Health Institution in Ethiopia: A Cross Sectional Study. *International Blood Research and Reviews*, 2(6):270-278
- Agarwal, R., Davis, J. and Smith, L. (2008). Serum albumin concentration is an important predictor of both baseline Hb and Epo sensitivity in chronic haemodialysis patients. Factors that improve serum albumin may also improve Hb in haemodialysis patients. (Serum Albumin Is Strongly Associated with Erythropoietin Sensitivity in Haemodialysis Patients. *Clinical Journal of the American Society of Nephrology*, 3(1): 98–104.
- Agbaji, O.O., Onu, A., Agaba, P.E., Muazu, M.A, Falang, K.D. and Idoko, J.A. (2011). Predictors of impaired renal function among HIV infected patients commencing highly active antiretroviral therapy in Jos, Nigeria. *Nigerian Medical Journal*, 52(3):182–185
- Amanzada, A., Goralczyk, A.D., Reinhardt, L., Moricon, P., Cameron, S. and Mihm,
   S. (2014). Erythropoietin rs1617640 G allele associates with an attenuated rise of serum erythropoietin and a marked decline of hemoglobin in hepatitis C patients undergoing antiviral therapy. *BioMed Center Infectious Diseases*, 14: 503.DOI: 10.1186/1471-2334-14-503.
- Ansari, T., Ali. L., Aziz, T., Ara, J., Liaquat, N. and Tahir, H. (2009). Nutritional iron deficiency in women of child bearing ages-what to do? *Journal of Ayub Medical College Abbottabad*, 21(3):17-20.
- Artunc, F. and Risler, T. (2007). Serum erythropoietin concentrations and responses to anaemia in patients with or without chronic kidney disease. *Oxford Journals Nephrology Dialysis Transplantation*, **22(10)**: 2900-2908.
- Aslinia, F., Mazza, J.J. and Yale, H.S. (2006). Megaloblastic anemia and other causes of macrocytosis. *Clinical Medicine and Research*, 4(3):236 241.

Audu, R.A., Akanmu, A.S., Mafe, A.G., Efienemokwu, C., Musa, A.Z.,Lemoha,
 E.Odunaike, M.I., Funso-Adebayo, E.O.Meshack, E and Idigbe, E.O. (2004).
 Changes in Serum Proteins and Creatinine levels in HIV Infected Nigerians.
 Nigerian Journal of Health
 and Biomedical Sciences, 3(2): 69-72.

#### Aukrust, P., Muller, F., Lien, E. Nordoy, I., Liabakk, N.B., Kvale, D., Espevik, T. and

- Froland, S.S. (1999). Tumor Necrosis Factor (TNF) System Levels in Human Immunodeficiency virus–Infected Patients during Highly Active Antiretroviral Therapy: Persistent TNF Activation Is Associated with Virologic and Immunologic Treatment Failure. *The Journal of Infectious Diseases*, 179:74-82.
- Bain, B. J. (1996). Ethnic and sex differences in total and differential white cell count and platelet count. *Journal of Clinical Pathology*, 49:664 - 666.
- Barlett, J. (2012). CD4 Cell Count and the Risk of AIDS or Death in HIV-Infected Adults on Combination Antiretroviral Therapy with a Suppressed Viral Load: A Longitudinal Cohort Study from COHERE. *Public Library of Science Medicine*, 9(3): e 1001194.

 Bastard, J.P., Soulié, C., Fellahi, S., Haim-Boukobza, S.C., Simon, A. and Katlama,
 C. (2012). Levels correlate with residual HI nd markers of immune dysfunction in treatment-controlled HIV- infected patients. *International Medical journal of Antiviral Therapy*, 17(5):915-9.

- Belperio, P.S. and Rhew, D.C. (2004). Prevalence and outcomes of anaemia in individuals with human immunodeficiency virus: a systematic review of the literature. *American Journal of Medicine*, 116 (Supplement. 7A):27S - 43S.
- Beggs, L.A. Yarrow, J.P., Conova, S.F., and Meuleman. J. R. (2014). Testoterone alters liver metabolite. American Journal of physiology, endocrinology and metabolism, 307 (5): E456 –E461.
- Beutler, E. and Waalen, J. (2006). The definition of anaemia: what is the lower limit of normal of the blood haemoglobin concentration? *Blood*, **107** (3):1747-1750.
- **Beutler, B. and Cerami, A. (1989).** The biology of cachectin /TNF- α primary mediator of the host response. *Annual Revision of Immunology*, **7**: 625-655.
- Beutler, E. and West, C. (2005). Haema tologic differences between Africans-Americans and Whites: The roles of iron deficiency and  $\alpha$  thalassemia on haemoglobin levels and mean corpuscular volume. *Blood*, **106(2)**:740-745.

Beverborg, G.N., Verweij, N. Klip, T., Van der Wal, H.h., Voors, A.V., Veldhuisen, D.J.V.,Gansevoort, R.T., Barkker, S.J.I., Harst, P.V. and Meer, P.V.D. (2015). Erythropoietin in the General Population: Reference Ranges and Clinical, Biochemical and Genetic Correlates. PLoS ONE 10(4): e0125215. doi: 10.1371/journal.pone.0125215.

- Blick, G., Khera, M., Bhattacharya, R.K. Kushner, H and Miner M.M. (2013). Testosterone Replacement Therapy in Men with Hypogonadism and HIV/AIDS: Results from the TRiUS Registry. *Postgraduate Medicine*, **125**(2): 19 - 29.
- Braamskamp, M. Dolman, K. and Tabbers, M. (2010). Protein-losing enteropathy in children. *European Journal of Pediatrics*, 169:1179-1185.
- Breen, E.C., Rezai, A.R., Nakajima, K., Beall, G.N., Mitsuyasu, R.T., Hirano, T., Kishimoto, T. and Martinez-Maza, O. (1990). Infection with HIV is
  - associated with elevated IL-6 levels and production. *Journal of Immunology*, **144**:480-484.
- Brian, C. and Olshaker, J.S. (1999). The C-reactive protein. *Journal of Emergency Medicine*, 17:1019-1025.
- Bush T.J., Walker H.K, Hall W.D and Hurst J.W. (1990). Clinical Methods: The History, Physical, and Laboratory Examinations. 3rd edition, Butterworths publication, Boston.
- Cappell, M. S. (1991). "Hepatobiliary manifestations of the acquired immune deficiency syndrome," *The American Journal of Gastroenterology*, **86**(1):1–15.
- Carol, J. and Ersieve, A.J. (1988). Erythropoietin assay and their use in the study of anaemia. *Control nephrology*, 66:54-62.
- Carrero, J.J., Qureshi, A.R., Nakashima, A., Arvers, S., Parini, P., Lindholm, B., Barany, P., Heimburger, O. and Stenvinkel, P. (2012). Prevalence and clinical implications of testosterone deficiency in men with end-stage renal disease. *Nephrology Dialysis Transplantation*, 27(2):709-15.

Castellares, C. P. Barreiro, L., Carbonero, M., Labarga, P., Vispo, Casado, R., Galindo, L., Garcia-Gasco, P., Garcia-Samaniego, J and Soviano, V. (2008). "Liver cirrhosis in HIV- infected patients: prevalence, etiology and clinical outcome," *Journal of Viral Hepatitis*, 15(3):165-172. Castro, G.K., Ward, J.W., Slutsker, L., Buehler, J.W., Jaffe, H.W. and Berkelman,
 R.L. (1993). Revised Classification System for HIV Infection and Expanded Surveillance
 Case Definition of AIDS Among Adolescents and Adults CDC.

Chan-yeung, M., Ferreira, P., Frohlich, J., Schulzer, M. and Tan, F. (1981). The effect of age, smoking and alcohol on routine laboratory tests. *American Journal of Clinical Pathology*.75 (3): 320 – 326.

Chaudhari, D.V., Jerker, S.C., Mehta, P.R. and Mania-Pramanik, J. (2013). Polymorphisms in major cytokine genes: A study among human immunodeficiency Virus-1serodiscordant couples in Mumbai, India. *Indian Journal of Medical Microbiology*, **31**(2): 166-172.

Chen, W., Wang, J., Abnet, C., Dawsey, S.M., fan, J., Yin, L., Yin, J., Taylor, P.R., Qiao, Y. and Freedman, N.D. (2015). Association between C - reactive protein, Incident Liver Cancer, and Chronic Liver Disease Mortality in the Linxian Nutrition Intervention Trials: A Nested Case–Control Study. *American Association for cancer research*, 24(2):322 - 422.

- Clinical and Laboratory Standards Institute (2008) Defining, establishing, and verifying reference intervals in the clinical laboratory; approved guideline-Third Edition. *National Committee for Clinical Laboratory Standards, Wayne, PA, USA C28-A3*, .28(30).
- Cloyd, M.W., Chen, J.J., Adegboyega, P. and Wang, L. (2001). How does HIV cause depletion of CD4 lymphocytes? A mechanism involving virus signaling through its cellular receptors. *Current Molecular Medicine*, 1 (5):545 550.
- **Coskun, A., Ceyhan, E., Inal, T.C., Serteser, M. and Unsal, I. (2013).** The comparison of parametric and nonparametric bootstrap methods for reference interval computation in small sample size groups. *Accreditation and Quality Assurance*, 18(1), 51–60.
- de Araújo, L.M., Lopes, F.M. and Garcia, L.V. (2014). Postoperative management of severe acute anemia in a Jehovah's Witness. *Transfusion*, **54**:1153-1157.

De Simone, V., Franzè, E., Ronchetti, G. Colantoni, A., Fantini, M.C. Fusco, D. D. Sica, G.S. MacDonald, T. T., Pallone, F., Monteleone, G. and Stolfi, C. (2015). Th17-type cytokines, IL-6 and TNF-α synergistically activate STAT3 and NF-kB to promote colorectal cancer cell growth. *Oncogene*, 34:3493-3497.

Delanghe, J.R., Bollen, M. and Beullens, M. (2008). Testing for recombinant erythropoietin. *American Journal of Haematology*, 83:237-241.

De Pablo-Bernal, R.S., Ruiz-Mateos,1.E., Rosado1, I., Dominguez-Molina, B., Alvarez-Ríos, A.I., Carrillo-Vico, A., De La Rosa, R., Delgado, J., Muñoz-Fernández,

**M. A., Leal, M. and Ferrando-Martínez, S.** (2014). TNF-α levels in HIV-infected patients after long-term suppressive cART persist as high as in elderly, HIV-uninfected subjects. *Journal of antimicrobial chemotherapy*, doi: 10.1093/jac/dku263.

Debevec, T., Keramidas, M.E., Norman, B., Gustafsson, T., Eiken, O. and Mekjavik,

- **I.B. (2012).** Acute short-term hyperoxia followed by mild hypoxia does not increase EPO production: *resolving the "normobaric oxygen paradox"*, **112(3):**1059 1065.
- **Devi, C.S., Satish, S. and Gupta, M. (2016).** Study of Haematological Profile in Human Immune Deficiency Virus Infection: Correlation with CD4 Counts. *Annals of Pathology and Laboratory Medicine*, **3(5)**, November Supplement.
- Diehl, A.M., Potter, J., Boitnott, J., Van Duyn, M. A., Herlong, H. F. and Mezey, E. (1984). Relationship between pyridoxal 5'-phosphate deficiency and aminotransferase levels in alcoholic hepatitis. *Gastroenterology*, 86: 632 636.
- **Dominici, S., Paolicchi, A., Corti, A., Maellaro, E. and Pompella, A. (2005).** Prooxidant reactions promoted by soluble and cell-bound gamma-glutamyltransferase activity. *Methods Enzymology*, **40**1:484–501.
- Drain, P., Kupka, R. and Mismanage, G. (2007). C-Reactive Protein Independently Predicts HIV-related Outcomes among Women and Children in a Resource-Poor Setting. *AIDS*, 21(15): 2067–2075.
- Duque, G. and Descoteaux, A. (2014). Macrophage Cytokines: Involvement in Immunity and Infectious Diseases. *Front Immunology*, **5**:491.

Dusingize, J.C., Hoover, D.R., Shi Q., Mutimura, E., Rudakemwa, E., Ndacyayisenga, V., Gakindi, L, Mulvihill, M., Sinayobye, J.D., Musabeyezu, E. and

- Anastos, K. (2015). Association of Abnormal Liver Function Parameters with HIV Serostatus and CD4 Count in Antiretroviral-Naive Rwandan Women. *AIDS Research and Human Retroviruses*, 31(7):723–730.
- Düzgün, S., Yıldırmak, Y. and Cetinkaya, F. (2005). Neutrophil hypersegmentation and thrombocytosis in children with iron deficiency anaemia. *The Turkish Journal of Pediatrics*, 47: 251-254.
- Ear, I. R. and Woteki, C.E. (1993). Iron deficiency anemia: recommended guidelines for the prevention, detection, and management among U.S. children and women of childbearing age. *Washington, DC: National Academy Press*.

- Edberg, M. and Hayes, B. (2015). Profile of HIV-Infected Hispanics with Pancytopenia. *International Journal of Environmental Research and Public Health*, 13(1):3
- Egrie, J.C. and Browne, J.K. (2001). Development and characterization of novel erythropoiesis stimulating protein (NESP). *British Journal of Cancer*, 84:3-10.
- **Ellaurie, M. and Rubinstein, A. (1995).** Elevated TNF-α in association with severe anaemia in HIV infection and mycobacterium avium intracellular infection. *Paediatric Haematology Oncology*, **12**:21-30.

Elliott, S., Lorenzini, T., Asher, S., Aoki, K., Brankow, D., Buck, L., Busse, L., Chang, D., Fuller, J., Grant, J., Hernday, N., Hokum, M., Hu, S., Knudten, A., Levin, N., Komorowski, K., Martin, F., Navarro, R., Osslund, T., Rogers, G., Rogers, N., Trail, G. and Egrie, J. (2003). Enhancement of therapeutic protein in vivo activities through glycoengineering. *Nature Biotechnology*, 21:414-421.

- Enawgaw, B., Alem, M., Melku, M., Addis, Z., Terefe, B. and Yitayew, B. (2015). Prevalence and associated risk factors of anemia among HIV infected children attending Gondar university hospital, Northwest Ethiopia: a cross sectional study. *BioMed Central Hematology*, 15:12.
- Erlandsen, E.J and Randers, E (2000). Reference interval for serum C-reactive protein in healthy blood donors using the Dade Behring N Latex CRP mono assay. *Scandinavian Journal of Clinical and Laboratory Investigation*. 60(1):37-43
- Eschbach, J.W., Egrie, J.C., Downing, M.R. Browne, J.K. and Adamson, J.W. (1987). Correction of the anaemia of end-stage renal disease with recombinant human erythropoietin. Results of a combined phase I and II clinical trial. *New England Journal of Medicine*, 316:73-78.
- Fabry, T.L. (1987). Mechanism of erythrocyte aggregation and sedimentation. *Blood*, 70(5):1572 1576.
- Ferede, G. and Wondimeneh, Y. (2013). Prevalence and related factors of anemia in HAART-naïve HIV positive patients at Gondar University Hospital, Northwest Ethiopia. *BIOMed Central Haematology*, 13:8. doi: 10.1186/2052-1839-13-8.
- Février, M., Dorgham, K. and Rebollo, A. (2011). CD4<sup>+</sup> T cell Depletion in Human Immunodeficiency Virus (HIV) Infection: Role of Apoptosis. *Viruses*, 3(5): 586 - 612.
- Fieldman, J.G., Goodwasser, P., Holman, S. DeHovitz, J. and Minkoff, H. (2003). Reactive protein is an independent predictor of mortality in women in HIV-1 infection. *Journal of acquired immune deficiency syndrome*, **32**:210 - 214.

- Foran, J.M., and Shammo, J.M. (2012). Clinical presentation, Diagnosis and Prognosis of Myelodysplastic Syndrome. *American Journal of Medicine*. 125(7):S6-S13.
- Ford, J. (2013). "Red blood cell morphology". International Journal of Laboratory Hematology. 35(3): 351-357.

Fosgerau, K., Galle, P., Hansen, T., Albrechtsen, A., Rieper, C.L., Pedersen, B.K., Larsen, L.K., Thomsen, A.R., Pedersen, O., Hansen, M.G. and Steensberg, A. (2009). Interleukin-6 autoantibodies are involved in the pathogenesis of a subset of type 2 diabetes. *Journal of endocrinology*, 204: 265 -273.

Gabriel, A., Kozek, S., Chiari, A., Fitzgerald, R., Grabner, C., Geissler, K., Zimpfer,
 M., Stockenhuber, F. and Bircher, N.G. (1998). High-dose recombinant human erythropoietin stimulates reticulocyte production in patients with multiple organ dysfunction syndromes. *Journal of Trauma*, 44:361-367.

Gatukui, D.K., Oyoo, G.O., Rajab, J., Kayima, J., Omonge, E. and Wanzala, P.
(2014). Serum erythropoietin in patients with anaemia on HAART attending the Kenyatta National Hospital, Comprehensive Care Centre. *East African Medical Journal of pathology*, vol.1 pp. 2-6.

- Gelaw, A., Shiferaw, Y. and Molla, R. (2013). Absolute lymphocyte counts as a surrogate marker for CD4+ cell count in monitoring of antiretroviral therapy, Northwest Ethiopia: retrospective evaluation. *Asian Pacific Journal of Tropical Disease*, 3(4):262-266.
- Gilbert, L.A., and Hemann, M.T. (2012). Context-specific roles for paracrine IL-6 in lymphomagenesis. *Genes and development*, 26:1758-1768.
- Giorgi, J.V. and. Hultin, L. E. (1990). Lymphocyte subset alterations and Immunophenotyping by flow cytometry in HIV disease. *Clinical Immunology Newsletter*, 10:55-61.
- Godnough, L., Rudinick, S. and Prince, T. (1989). Increased preoperative collection of autologous blood with recombinant human erythropoietin therapy. *New England Journal of Medicine*, **321**:1163-1168.
- Guidelines for Conduct of Clinical Trials in Kenya, Pharmacy and Poisons Board, September 2016. Registrar Pharmacy and Poisons Board, P. O. Box 27663-00506 Nairobi, Kenya Tel: +254 20 3562107 +254 733 884411 / 720 608811
- Guillen, M.I., Gomez-Lechon, M.J., Nakamula, T. and Castell, J.V. (1996). The Hepatocyte Growth Factor Regulates the Synthesis of Acute-Phase Proteins in Human Hepatocytes: Divergent Effect on Interleukin-6 - Stimulated Genes. *Hepatology*, 23(6):1345 -1352.

Guimarães, M.M., Greco, D.B. & Figueiredo, S.M. (2008). High-sensitivity Creactive protein levels in HIV-infected patients treated or not with antiretroviral drugs and their correlation with factors related to cardiovascular risk and HIV infection. *Atherosclerosis*, 201(2):434-439.

Greenberg, P. Sun, Z., Miller, K., Bennett, J.M., Tallman, M.S., Dewald, G., Paietta,

- E., Jagt, R.V., Houston, J., Thomas, M.L., Cella, D. and Rowe, J.M. (2009).Treatment of myelodysplastic syndrome patients with erythropoietin with or without granulocyte colony-stimulating factor: results of a prospective randomized phase 3 trial by the Eastern Cooperative Oncology Group (E1996). *Blood*, 114(12):2393–2400.
- Grossi, A., Balestri, F. and Santini, S. (2007). Darbepoetin alpha in the treatment of cancer chemotherapy-induced anemia. *Therapeutic Clinical Risk, Management*, **3**(2):269-275.

Grote, B.N., Verweij, N., Klip, I.T., Van der Wal, H.H., Voors, A.A., Veldhuisen, D.J., Gansevoor, R.T., Bakker, S.J.L., Harst, P.V. and Meer, P.V. (2015). Erythropoietin in the general population: Reference ranges and clinical, biochemical and genetic correlates. *PLoS ONE*, **10**(4) e0125215. doi:10.1371/journal.pone.0125215.

- Haase, V.H. (2010). Hypoxic regulation of erythropoiesis and iron metabolism. *American journal of renal physiology*, **299**(1): F1–F13.
- Hallberg, L. (1988). Iron balance in pregnancy. In: Berger H. Vitamins and minerals in pregnancy and lactation. *New York, Raven Press*, 115-127.
- Handan, C., Midraci, T., Mehmet, C. Bilge, M., Ahme, B., and Erdal, M. (2009).
  "Markedly elevated serum alkaline phosphatase level in an uncomplicated pregnancy" *The Journal of Maternal-Fetal & Neonatal Medicine: The Official Journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstetricians*, 22(8):705-707.
- Henry, D.H., Bowers, P., Romano, M.T. and Provenzano, R. (2004). Epoetin alfa. Clinical evolution of a pleiotropic cytokine. *Archives of Internal Medicine*, 164(3):262-276.
- Hellstrom-lindberg, E., Negrin, R., Stein, R., Krantz, S. and Lindberg, G. (1997). Erythroid response to treatment with G-CSF plus erythropoietin for the anaemia patients with myelodysplastic syndromes: proposal for a predictive model. *British Journal of Haematology*, 99:344 -351.

Henrik, L., Marcus, D., Jette, V., Peter, G. C., Klaus, B., Peter, S., Klarlund, P.B. (1996). Increased circulating levels of interleukin- 6 in HIV-seropositive subjects. *Journal of Acquired Immunodeficiency syndromes and Retrovirology*, 13(1):93-94.

- Hoffbrand, A.V., Catovsky, D. and Tuddenham, E.G. (2005). Postgraduate haematology; 4<sup>th</sup> edition. *Blackwell publication*, Pp. 352- 353.
- Huang, C.R. (1987). Thixotropic properties of whole blood from healthy human subjects. *Biorheology*, 24(6):795-801.
- Huibers, M.H., Bates, I., McKew, S. and Allain, T.J. (2020). Severe anaemia complicating HIV in Malawi; Multiple co-existing aetiologies are associated with high mortality. *PLoS ONE* 15(2):e0218695.
- Hussain, T., Abbas, S.W. and Zareen, F.S. (2008). Reactive Thrombocytosis following acute infection and pancytopenia. *Pakistan Armed Forces Medical Journal*, 58 (2):219-22.
- Hyder, M., Hasan, M. and Mohieldein, A. (2013). Comparative Levels of ALT, AST, ALP and GGT in Liver associated Diseases. *European Journal of Experimental Biology*, 3(2):280-284.
- **Ibeh, B., Omodamiro, O., Ibeh, U. and Habu, J.B. (2013).** Biochemical and haematological changes in HIV subjects receiving winniecure antiretroviral drug in Nigeria. *Journal of Biomedical Science*, **20(1)**:73.
- Ikunaiye, N.Y., Denue, B.A. Aina, B.A., Aderemi-Williams, R. and Rawizza, H.E. (2018). Incidence of anaemia among HIV-infected patients treated with zidovudine- containing antiretroviral therapy in Northeastern Nigeria. Annals Ibadan Postgraduate Medicine. 16(2): 115–124.

#### International Committee for Standardization in Hematology. (1978).

Recommendations for reference method for hemoglobinometry in blood and specifications for international hemioglobicyanide, reference preparation. *Journal of Clinical Pathology*, **31** antiretroviral therapy in Northeastern Nigeria.

Jacobs, R.H., Cornbleet, M.A., Vardiman, J.W. Larson, R.A., Le Beau, M.M. and Rowley, J.D. (1986). Prognostic implications of morphology and karyotype of primary myelodysplastic syndromes. *Blood*, 67:1765-1772.

- Jacob, A.E. (2016). Complete Blood Cell Count and Peripheral Blood Film, Its Significant in Laboratory Medicine A Review Study. American Journal of Laboratory Medicine., 1(3):34 – 57.
- Jain, S., Gautama, V. and Naseem, S. (2011). Acute-phase proteins: As diagnostic tool. *Journal of Pharmacy and Bioallied Sciences*, 3(1):118-127.

#### Jamison, D.T., Feachem, R.G., Makgoba, M.W., Bos, ER, Baingana, F.K., Hofman,

K.J. and Rogo, K.O. (2006). Disease and mortality in Sub-Saharan Africa. 2nd edition, Washington (DC). *World Bank Chapter 1*.

- Jelkmann, W. (2013). Physiology and Pharmacology of Erythropoietin. *Transfusion Medicine and Haemotherapy*, 40(5):302-309.
- Jiang, S., Jiang, D., and Tao, Y. (2013). Role of gamma-glutamyltransferase in cardiovascular diseases. *Experimental Clinical cardiology*, 18:53-56.
- Jong, M.A., Witte, L., Oudhoff, M.J. Gringhuis, S.I., Gallay, P. and Geijtenbeek, T.B.H. (2008). TNF-α and TLR agonists Increase susceptibility to HIV-1 transmission by human Langerhans cells exvivo. *Journal of clinical investigation*, **118**(10):3440-3452.
- Kaewketthong, P., Bunyaratvej, A. and Barusrux, S. (1992). Different cell volume with high target cell population between liver disease and homozygous hemoglobin E. *Journal of the Medical Association of Thailand*, **75(1):**228-232.
- Kaferle, J. and Strzoda, C.E. (2009). Evaluation of macrocytosis. *American Family Physician*, **79**(3):203 208.
- Kalyani, P. and Jamil, K. (2015). A study on biochemical facet of anemia in cancers: A strong link between erythropoietin and tumor necrosis factor alpha in anaemic cancer patients. *The Indian Journal of cancer*, **52**(1):127 132.
- Kao, T.W., Chou, C.H., Wang, C.C., Chou, C.C., Hu, J. and Chen, W.L. (2012).
  "Associations between serum total bilirubin levels and functional dependence in the elderly". *Internal Medicine Journal*, 42(11):1199 207.

Karita, E., Ketter, N., Price, M.A., Kayitenkore, K., Kaleebu, P., Nanvubya, A.,
Anzala, O., Jaoko, W., Mutua, G., Ruzagira, E., Mulenga, J., Sanders, E.J. and
Mwangome, M. (2009). CLSI-Derived Hematology and Biochemistry Reference
Intervals for Healthy Adults in Eastern and Southern Africa. *PLoS ONE*, 4(2): e4401.

Kasthuri, A., Sanjeevan, S. and Kar, P. (2006). A study of hematological manifestations of HIV Infection. Indian Journal Sex Transm Dis 27:9-16.

Kazama, I., Endo, Y., Toyama, H., Ejima, Y. and Kurosawa, S. (2011). Compensatory Thrombopoietin Production from the Liver and Bone Marrow Stimulates Thrombopoiesis of Living Rat Megakaryocytes in Chronic Renal Failure. *Nephron Extra*, 1:147-156.

Khanna, K.V., Xiao-Fang, X.F., Ford, D.H. Ratner, L. Hildreth, J and Markham,
R. (2017). Differences Among HIV-1 Variants in Their Ability to Elicit Secretion of TNFa. The Journal of Immunology, 164:1408-1415.

- Kharsany, A.B.M. and Karim, Q. A. (2016). HIV infection and AIDS in sub-Saharan Africa: Current Status, Challenges and Opportunities. *Open AIDS Journal*, 10: 34 – 48.
- Kim, W., Flamm, S., Bisceglie, A. and Bodenheimer, H.C. (2008). Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. *Hepatology*, 47(4):1363 - 1370.
- Kiss, Z., Elliot, S., Jedynasty, K., Tesar, V. and Szegediet, J. (2010). Discovery and basic pharmacology of erythropoiesis-stimulating agents (ESAs), including the hyperglycosylated ESA, darbepoetin alfa: an update of the rationale and clinical impact. *European Journal of Clinical Pharmachology*, 66:330 – 340.

Korum, K., Addae, M., Ocran, J., Adu-Amankwah, S., Rogers, W.O. and Nkrumah,
 F.K (2007). Population based reference intervals for common blood haematological and biochemical parameters in the Akuapem North district. *Ghana Medical Journal*, 41(4):160–166.

Kreuzer, K.A. and Rockstroh, J.K. (1997). Pathogenesis and pathophysiology of Anaemia in HIV infection. *Annals of Haematology*, 175:179-187.

Kupka, R., Msamanga, G.I., Mugusi, F., Petraro, P., Hunter, D.J. and Fawzi, W.W.
(2007). Iron status in an important cause of anaemia in HIV infected Tanzanian women, but is not related to Accelerated HIV Disease Progress. *Journal of Nutrition*. 137(10):2317-2323.

- Kyeyune, R., Saathoff, E., Ezeamama, E.A., Löscher, T., Fawzi, W. and Guwatudde, D. (2014). Presence and correlates of cytopenias in HIV infected adults initiating highly active antiretroviral therapy in Uganda. *BioMed Cental Infectious Diseases*, 14:496 https://doi.org/10.1186/1471-2334-14-496.
- Kyi, A.S. and Sidibem, M. (2012). United Nations Acquired Immunodeficiency World AIDS Day Report, Pp. 30-34.
- Langford, S., Ananworanich, J. and Cooper, D. (2007). Predictors of Disease Progression in HIV Infection: A review. *AIDS Research and Therapy*, **4**:11.

Lau, B., Sharrett, A.R., Kingsley, L.A., Post, W., Palella, F.J., Visscher, B. and Grange, S.J, (2006). C-reactive protein is a marker for human immunodeficiency virus disease progression. C - reactive protein is a Marker for Human Immunodeficiency Virus Disease Progression? *Archives of Internal Medicine*, 166(1):64-70.

Lee, D.E., Son, W., Ha, B.J. Oh, M.S. and Yoo, O.J. (2006). The prolonged half-lives of new erythropoietin derivatives via peptide addition. *Biochemical and Biophysical Research Communication*, 339(1):380-385.

- Levin, G.Y. and Egorihina, M.N. (2013). The role of oxidized albumin in blood cell aggregation disturbance in burn disease. International Journal of Burns Trauma, 3(2):115-121.
- Levy, J.A. (1993). Pathogenesis of Human Immunodeficiency Virus. *Microbiology* and Molecular Biology Reviews, 57(1):183 – 289.

#### Lin, F.K., Suggs, S., Browne, J.K., Smalling, R., Egrie, J.C., Chen, K.K., Fox,

**G.M., Martin, F. and Stabinsky, Z. (1985)**. Cloning and expression of the human erythropoietin gene. Proceedings of the National Academy of Sciences of the United States of America, **82(22)**:7580-7584.

Lugada, E.S., Mermin, J., Kaharuza, F., Ulvestad, E., Were, W., Langeland, N.,

- Asjo, B., Malamba, S. and Downing, R. (2004). Population-based hematologic and immunologic reference values for a healthy Ugandan population. *Clinical Diagnosis Laboratory Immunology*, 11:29-34.
- Lum, G. and Gambino, S.R. (1972). "Serum gamma-glutamyl transpeptidase activity as an indicator of disease of liver, pancreas, or bone". *Clinical Chemistry*, 18 (4):358-362.

Luna, J., Moon, Y., Liu, K., Spitalnik, S., Paik, M.C., Cheung, K., Sacco, R.L. and Elkind, M.S.V. (2014). High sensitivity C-Reactive Protein and Interleukin-6 Dominant Inflammation and Ischemic Stroke Risk: The Northern Manhattan Study. *Stroke*, 45(4): 979 - 987.

- Madaan, G.B., Jairajpuri, Z.S., Hajini, F.F. and Jetley, S. (2015). Postoperative thrombocytosis: An unusual case report. *International Journal of Applied Basic Medical Research*, 5(3):225-227.
- Marina, N., Raquel, L, Juan, M. L., Luz, M. and Vincent, S. (2001). "Risk factors for severe hepatic injury after introduction of highly active antiretroviral therapy," *Journal of Acquired Immune Deficiency Syndromes*, 27(5): 426– 431.
- Marshall, P.N., Bentley, S.A. and Lewis, S.M. (1978). Staining properties and stability of a standardized Romanowsky stain. *Journal of clinical pathology*, **31**:280-82.
- Masaisa, F., Gahutu, J.B., Mukiibi, J., Delanghe, J. and Philippé, J. (2011). Anaemia in Human Immunodeficiency Virus infection and uninfected women in Rwanda. American Journal of Tropical Medicine and Hygiene, 84:456-460.
- Mathews, S.E., Srivastava, D., Yadav, R.B. and Sharma, A. (2013). Association of haematological profile of human immunodeficiency virus-positive patients with clinicoimmunologic stages of the disease. *Journal of laboratory physicians*, **5**(1): 34-37.

- Mawani, M., Ali, S.A., Bano, G. and Ali, S.A. (2016); Iron Deficiency Anemia among Women of Reproductive Age, an Important Public Health Problem: Situation Analysis.
- McArdle, P., Whitcomb, B. and Tanner, K. (2012). Association between bilirubin and cardiovascular disease risk factors: using Mendelian randomization to assess causal inference. *Biomed Central Cardiovascular Disorders*, DOI: 10.1186/1471-2261-12-16.

Mecha J.O., Kubo, E.N. and Nganga, L.W. (2016). Trends in clinical characteristics and outcomes of Pre-ART care at a large HIV clinic in Nairobi, Kenya: a retrospective cohort study. *AIDS Research Therapy*.13: 38.

Meidani, M., Rezaei, F., Maracy, R.M., Avijgan, M. and Tayeri, K. (2012). Prevalence, severity, and related factors of anemia in HIV/AIDS patients. *Journal of Research in Medical Sciences*, **17**(2): 138–142

 Menard, D., Mandeng, M.J., Tothy, M.B., Kelembho, E.K., Gresenguet,
 G. and Talarmin, A. (2009). Immunohaematological reference ranges for adults from the Central African Republic. *Clinical Diagnosis Laboratory Immunology*, 10(3): 443-445.

- Merlot, A.M, and Richardson, D.R. (2014). Unraveling the mysteries of serum albumin –more than just a serum protein. *Frontiers in Physiology*, 5:299.
- Micali, S. (1993). Mechanism for the T4 lymphopenia of AID. *Immunology*, 90: 10982-10983.

Mittelman, M., Zeidman, A., Fradin, Z., Magazanik, A., Lewinski, U.H. and Cohen, A. (1997). Recombinant human erythropoietin in the treatment of multiple myelomaassociated anaemia. *British Journal of* Haematology, 98:204-210.

- Moir, S. and Fauci, A. (2009). B cells in HIV infection and disease. *Nature Reviews-Immunologyogy*, 9(4): 235–245.
- Monzote, L. and Siddiq, A. (2011). Drug Development to Protozoan Diseases. *Open Medicinal Chemistry Journals*, 5: 1-3.
- Morceau, F., Dicato, M. and Diederich, M. (2009). Mediators of Inflammation Pro-Inflammatory Cytokine-Mediated Anemia: Regarding Molecular Mechanisms of Erythropoiesis. *Journal of Mediators of Inflammation* 1-11.

Mugisha, J.O., Donegan, K., Fidler, S., Ramjee, G., Hodson, A., Dunn., D.T., Porter, K. and Kaleebu, P. (2012). Mean Corpuscular Volume as a Marker for Adherence to Zidovudine-Containing Therapy in HIV-Infected

Adults. Open AIDS Journal, 6:45-52.

- Mugisha, J.O., Seeley, J. N.D. and Kupper, H. (2016). Population based haematology Ranges.in Rural South-West Uganda. BMC Research notes, 9:433.
- Munyazesa, E., Emile, I., Mutimura, E. and Hoover, D.R. (2012). Assessment of haematological parameters in HIV-infected and uninfected Rwandan women: a cross- sectional study. *British Journal of Medicine*, 2: e001600 doi: 10.1136/bmjopen-2012-00160.
- Murphy, W.G., (2014). The sex difference in haemoglobin levels in adults Mechanisms, causes, and consequences. *Blood Reviews*, 28(2):41-47.
- Mutabazi, J.C., Zarowsky, C, and Trottier, H. (2017). The impact of programs for prevention of mother-to-child transmission of HIV on health care services and systems in sub-Saharan Africa *A review*. *Public Health Reviews*. **38**: 28.
- Naicker, S. (2003). End-stage renal disease in sub-Saharan and South Africa. *Kidney International*, 63:5119-5122.
- Naif, M.H. (2013). Pathogenesis of HIV Infection. Infectious Disease Reports, 5(1):e6
- National AIDS Control Council, (2016). National AIDS & STI Control Programme, www.nascop.or.ke. *Pp.* 3 253.
- Naveen, R., Akshata, K., Pimple, S. and Chaudhari, P. A. (2016). Review on albumin as drug carrier in treating different diseases and disorders. *Der Pharmacia Sinica*, 7(1):11-15.
- Naeim, F. Rao, N.P. and Phan, T.R. (2018). Atlas of Haematology; Morphology, Immunophenotype, *Cytogenetics and Molecular approaches*. 2<sup>nd</sup> edition. Pp. 871-884.
- Nemeth, E., Rivera, S., Gabayan, V., Keller, C., Taudorf, S., Pedersen, B.K. and Ganz, T. (2004). "IL-6 mediates Hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin." *Journal of Clinical Investigation*, 113(9):1251-1253.
- Netto, I., Borgaonkar, K. and Lobo, R. (2009). Aminotransferase profile in HIV positive patients. *Indian Journal of Sexually Transmitted Diseases and AIDS*, **30**(2): 121.
- Ngala, R.A., Opoku, D. and Asare, G. (2015). Effects of HIV Infection and Highly Active Antiretroviral Therapy (HAART) on the Liver of HIV Patients. *Trends in Medical Research*, **10**: 1-11.

**Obirikorang, C. and Yeboan, F.A. (2009).** Blood haemoglobin measurement as a predictive indicator for the progression of HIV/AIDS in resource – limited setting. *Journal of Biomedical Science*, **16**:1-7.

Odhiambo, C., Omolo, P., Oyalo, B., Williamson, J., Kinuthia, J., Matemo,

- D., Drake, A., John-Stewart, G. and Zeh, C. (2017). Establishment of reference interval during normal pregnancy through six months postpartum in Western Kenya. *PLoS ONE* 12(4): e0175546.
- Ogba, O.M., Abia-Bassey, L.N. and Epoke, J. (2013). Haematological Profile of HIV Infected Patients with Opportunistic Respiratory Mycoses in Relation to Immune Status–A Hospital Based Cohort from Calabar, Nigeria. *Tropical Medicine & Surgery*. 1:122.
- **Ogedegbe, A.O. and Sulkowski, S.M. (2003)**. Antiretroviral-associated liver injury. *Clinics in Liver Disease*, **7**: 475 499.
- Oliveira, O.C., Oliveira, R.A. and Souza, L.R. (2011). Impact of antiretroviral therapy on occurrences of macrocytosis in patients with HIV/AIDS in Maringá, State of Paraná. *Revista da Sociedade Brasileira de Medicina Tropical*. 44(1):26-29.
- Olu-Taiwo, A., Akanmu, S., Omusu, O. and Ogunro, S. (2013). Estimation Of Serum Erythropoietin Levels in Anaemic HIV Infected Patients in Lagos Nigeria. *Blood*, 122: 4656.
- Ownby, R.L., Kumar, A.M., Fernandez, J.B., Moleon-Borodowsky, I., Gonzalez, L., Eisdorfer, S., Waldrop-Valverde, D. and Kumar, M. (2009). Tumor Necrosis Factor- alpha Levels in HIV-1 Seropositive Injecting Drug Users. *Journal of Neuroimmune Pharmacology*, 4(3): 350–358.
- Özcan, A., Toraman, A., Çolak, A., Yazgan, H., Demirdöven, M., Yokuş, O. and Gürel, A. (2011). Evaluation of leucocyte and its subgroups in iron deficiency anemia. *International Journal of Medicine and Medical Sciences*, **3**(5): 135-138.
- Paiardini, M. and Müller-Trutwin, M. (2013). HIV-associated chronic immune activation. *Immunological Review*, 254(1): 78-101.
- Panwar, A., Sharma, S.C., Kumar, S. and Sharma, A. (2016). A study of anemia in human immunodeficiency Virus patients: Estimating the prevalence, analyzing the causative effect of nutritional deficiencies and correlating the degree of severity with CD4 cell counts. *Medical Journal*, 9(3): 312-318.

- Parinitha, S.S. and Kulkarni, H.M. (2012). Haematological changes in HIV infection with correlation to CD4 cell count. *Australia Medical Journal*, 5(3):157-162.
- Pepys, M.B. and Hirschfield, G.M. (2003). C-reactive protein: a critical update. Journal of Clinical Investigation, 111(12):1805-1812.
- Plat, E.J., Durnin, J.P. and Kabat, D. (2005). Kinetic factors control of cell entry, efficacies of entry inhibitors and mechanism of adaptation of human immunodeficiency virus. *Journal of Virology*, **79**: 4347-56.
- Pompella, A., Meden, M., Passino, C. and Paolicchi, A. (2004). The significance of serum γ-glutamyltransferase in cardiovascular diseases. *Clinical Chemistry Laboratory Medicine*, 42: 1085-1091.

Popa, C., Netea, M.B., Van Riel, P.L., van der Meer, J.W.M. and. Stalenhoef, A.F.H.
(2007). The role of TNF-α in chronic inflammatory conditions, intermediary metabolism, and cardiovascular risk. *The Journal of Lipid Research*, 48: 751-762.

Price, C. and Alberti, K. (1979). Biochemical assessment of liver function. Liver and biliary diseases-pathophysiology, diagnosis, management. *London: W. B. Saunders*, 381-416.

Prutchi-Sagir, S., Golishevsky, N., Oster, H.S. Katz, O., Cohen, A., Naparstek, E., Neumann, D., and Mittelman, M. (2006). Erythropoietin treatment in advanced multiple myeloma is associated with improved immunological functions: could it be beneficial in early disease? *British Journal*, 135:660-672.

Quintanar, J.L. and Guzmán-Soto, I. (2013). Hypothalamic neurohormones and immune responses. *Frontiers in integrated neuroscience*, **7:**56.

- Quest Diagnostics Nichols institute. (2013). Tumor necrosis factor. Mayo Medical Clinic test catalogue. *FFtum Clinical*, Pp. 1-2.
- Rahman, M.M., Giti, S., Islam, M.S. and Rahman, M.M. (2014). Haematological Changes in Peripheral Blood of HIV –Infected Persons with Correlation to CD4 Cell Count. *Journal of Bangladesh College of Physicians and Surgeons*, 32:130-136.

Rarick, M.U., Loureiro, C., Groshen, S., Sullivan-Halley, J., Gill, P.S., Bernstein-Singer, M. and Levine, A.M. (1992). Serum erythropoietin titers in patients with human immunodeficiency Virus infection and anaemia. *Journal of Acquired Immune Deficiency Syndrome*, 5:424-442

- Redd, A.D., Eaton, K.P., Kong, X., Laeyendecker, O., Lutalo, T., Wawer, M.J., Gray,
- R.H., Serwadda, D. and Quinnon, T.C. (2010). C-reactive protein levels increase during HIV-1 disease progression in Rakai, Uganda despite the absence of microbial translocation. *Journal of Acquired Immune Deficiency* Syndrome, 54(5):556 -559.
- Redig, A. and Berliner, N. (2013). Pathogenesis and clinical implications of HIV-related anemia. *American Society of Hematology, the education programme*, 377-381.
- Richman, D.D., Fischl, M.A. and Grieco, M.H. (1987). The toxicity of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. A double-blind, placebo- controlled trial. *New England Journal of Medicine*, 317:192-197.
- Ridker, P.M., Hennekens, C.H., Buring, J.E. and Rifai, N. (2000). C- reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *New England Journal of Medicine*, 342(12):836–483.
- Rocchetta, F., Mister, M., Mele, C., Cassis, P., Noris, M., Remuzzi, G. and Aiello, S. (2011). Erythropoietin enhances immunostimulatory properties of immature dendritic cells. *Immunology*, 165(2): 202–210.
- Ruchira, V., Diazzi, C., Santi, D., Brigante, G., Ansaloni, A., Decaroli, M.C., De Vincentis, S., Stentarelli, C., Zona, S. and Guaraldi, G. (2015). Low testosterone is associated with poor health status in men with human immunodeficiency virus infection: a retrospective study. *Andrology*, 3:298-308.

Ruttmann, E., Brant, L.J., Concin, H., Diem, G., Rapp, K., Ulmer, H., Vorarlberg Health Monitoring and Promotion Program Study Group. (2005). Gammaglutamyltransferase as a risk factor for cardiovascular disease mortality: an epidemiological investigation in a cohort of 163,944 Austrian adults. *Circulation*, **112**:2130-2137.

Ryst, E.V., Kotze, M., Joubert, G., Steyn, M., Pieters, H., van der Westhuizen,
M., van Staden, M. and Venter, C. (1998). Correlation among Total Lymphocyte Count, Absolute CD4+ Count, and CD4+ Percentage in a Group of HIV-1-Infected South African Patients. *Cornea Journal of Acquired Immune Deficiency Syndromes & Human Retrovirology*, 19(3):238-244.

Saathoff, E., Schneider, P., Kleinfeldt, V., Geis, S., Haule, D., Maboko, L., Samky, E., de Souza, M., Robb, M. and Hoelscher, M. (2008). Laboratory Reference values for Healthy Adults from Southern Tanzania. *Tropical Medicine and International Health*, 13(5): 612- 625. Sade-Feldman, M., Kanterman, J., Shalom, E., Elnekave, M., Horwitz, E. and Baniyash, M. (2013). Tumor Necrosis Factor-A Blocks Differentiation and Enhances Suppressive Activity of Immature Myeloid Cells during Chronic inflammation. *Journal of immunity*, 38(3):541 - 554.

Santis, G.C., Brunetta, D.M., Vilar, F.C., Brandão, B.A., Muniz, R.Z.A., Nogueirade, G.M., Amorelli-Chacel, L.E., Covas, D.T. and Machado, A.A. (2011). Hematological abnormalities in HIV-infected patients. *International Journal of Infectious Diseases*, 15(12): e808-e811.

- Saverino, A., Pescarmona, G.P. and Boelaert, J.R. (1999). Iron metabolism and HIV infection: reciprocal interactions with potentially harmful consequences. Cell Biochemistry Function, 17:279-287.
- Scarpino, M., Santoro, M. and Pellicano, G. (2015). HIV infection and kidney disease: literature review. *Infectious Disease Tropical Medicine*, 1(4): E195.

Shivakoti, R., Yang, W-T., Gupte, N., Berendes, S., Rosa, A.L. Cardoso, S.W., Mwelase, N., Kanyama, C., Pillay, S., Samaneka, W., Riviere, C., Sugandhavesa, P., Santos, B., Poongulali. S., Tripathy, S., Bgerollin, R.C., Currier, J.S., Tang, A.M., Semba, R.D. and Christian, P. (2015). Concurrent Anemia and Elevated

C-Reactive Protein Predicts Clinical Treatment Failure, Including Tuberculosis, After Antiretroviral Initiation. *Clinical Infectious Disease*, **61**: 102 – 110.

Schuett, H., Luchtefeld, M., Grothusen, C., Grote, K. and Schieffer, B. (2009). Interleukin - 6 and its signaling in antheroscerosis. *Thrombosis and haematology*, 2(2);215-222.

Sedlak, W.T., Saleh, M., Higginson, D.S., Paul, B.D., Juluri, K.R. and. Snyder, S.H. (2009). Bilirubin and glutathione have complementary antioxidant and cytoprotective roles. *Proceedings of the National Academy of Sciences of the United States* of America, 106(13):5171-5176.

- Seiyaku, C. and Hiroshi, S.K. (2005). Remedies for protozoan diseases. *Publication number*, US 0148503A1 Pp.1.
- Semba, D.R. and Gray, E.G. (2001). Pathogenesis of Anaemia During Human Immunodeficiency Virus Infection. Journal of Investigative Medicine, 49(3):225-239.
- Sharma, K., Sharma, S.B., Pukhta, I.A., Sharma, A.B. and Salaria, A.O. (2013). Increased preoperative collection of autologous blood with recombinant human erythropoietin therapy in tertiary care hospitals of Jammu. *Asian Journal of Transfusion Science*, 7(1):42-47.

- Sinclair, A. (2013). Erythropoiesis stimulating agents: approaches to modulate activity. *Biologics*, 7:161–174.
- Sinert, R. and Spektor, M. (2005) Clinical Assessment of Hypovolemia. Annals of Emerging Medicine. 45:327-329.
- Sipahi, T., Tavil, B. and Unver, Y. (2002). Neutrophil hypersegmentation in children with iron deficiency anemia. *Journal of Pediatric Haematology and Oncology*, 19(4):235-238.
- Sorbi, D., Boynton, J. and Lindor, K. (1999). The ratio of aspartate aminotransferase to alanine aminotransferase: potential value in differentiating Nonalcoholic steatohepatitis from alcoholic liver disease. *American Journal of Gastroenterology*, 94: 1018–1022.
- Sparling, E.A., Nelson, C.L., Lavender, R. and Smith, J. (1996). The use of erythropoietin in the management of Jehovah 's witnesses who revision total hip arthroplasty. *Journal of bone and joint surgery*, 78:1548-52.
- Speight, C. and Kenneth, C. (2004). Ministry of Health, National AIDS and STD Control Programme, "Kenyan National Clinical Manual for ARV providers" 1<sup>st</sup> edition; NASCOP, Nairobi, pp. 3-20.
- Spinowitz, B.S. and Pratt, R.D. (2006). Epoetin Delta 2002 Study Group. Epoetin delta is effective for the management of anaemia associated with chronic kidney disease. *Current Medical Response Opinion*, 22:2507-2513.
- Srirangan, S. and Choy, E. (2010). The Role of Interleukin- 6 in the Pathophysiology Of Rheumatoid Arthritis. *Therapeutic Advances Musculoskeletal Disease*, 2(5): 247 -256.
- Stovall, T.G. (2001). Clinical experience with epoetin alfa in the management of haemoglobin levels in orthopaedic surgery and cancer. Implications for use in gynaecologic surgery. *Journal of Reproduction Medicine*, 46:531-538.
- Sriharsha, D., Kalani, R. L., Bradley, B.C. Alfred K.C., Tom G., and Srinivasan, B.
   (2011). "Serum alkaline phosphatase levels Associated with elevated serum C-reactive protein in chronic kidney disease." *Kidney International*, 79(2): 28-233.
- Subbiah, S.K. (2017). Haematological and CD4<sup>+</sup> T cells reference ranges in healthy adult populations in Gojjam zones in Amhara region, Ethiopia. *PLoS One*. 12(7): e0181268

- Suliivan, p., Hanson, D., Richardson, J. and Brooks, J.T. (2011). Trends in the Treatment of Anemia Using Recombinant Human Erythropoietin in Patients with HIV Infection. *Open AIDS Journal*, 5: 113-118.
- Tamir, Z., Seid, A. and Haileslassie, H. (2019). Magnitude and associated factors of cytopenias among antiretroviral therapy naïve Human Immunodeficiency Virus infected adults in Dessie, North east Ethiopia. PLoS ONE 14(2):e0211708.

Tembe, N., Joaquin, O., Alfai, U., Sitoe, N., Viegas, E., Macovela, E., Gonçalves, E.,

Osman, N., Andersson, S., Jani, I. and Nilsson, C. (2014). Reference values for clinical Laboratory Parameters in Young Adults in Maputo, Mozambique. *PLoS ONE* 9(5): e97391. doi: 10.1371/journal. pone.009. Sullivan

Thapa, B. and Walia, A. (2007). Liver Function Tests and their Interpretation. *Indian Journal of Pediatrics*, 74(7): 663-671.

Treacy, M., Lai, L., Costello, C. and Clark, A. (1987). Peripheral blood and Bone marrow abnormalities in HIV related disease. *British Journal of Haematology*, 65(3):289 – 294.

Tsiakalos, A., Routsias, J.G., Kordossis, T., Moutsopoulos, H.M., Tzioufas, A.G. and Sipsas, N.V. (2011). Fine epitope specificity of anti-erythropoietin antibodies reveal molecular mimicry with HIV-1 p17 protein: a pathogenetic mechanism for HIV-1-related anemia. *Journal of Infectious Diseases*, 204(6):902 - 911.

- Unger, E.F., Thompson, A.M., Blank, M.J. and Temple, R. (2010). Erythropoiesis Stimulating agents - time for a reevaluation. New England Journal of Medicine, 362:189-192.
- VanWagner, L. and Green, R. (2015). Evaluating Elevated Bilirubin Levels in Asymptomatic Adults. *Journal of the American Medical Association*, 313(5): 516 -517.
- Veda, P. (2012). Evaluation of macrocytosis in routine haemograms. *Indian Journal of Hematology and Blood Transfusion*, 29(1):26-30.

- Vijayan, V.K.K., Karthigeyani, P. K., Tripathi, P.S. and Hanna, E.L. (2017). Pathophysiology of CD4 + T - cell Depletion in HIV-1 and HIV-2 infections. *Frontiers in immunology*, **8**:580.
- Vishnu, P. and Aboulafia, D.M. (2015). Haematological manifestations of human immune deficiency virus infection. *British Journal of Haematology*, 171(5):695–709.
- Waithaka, S.K., Njagi, E.N., Ngeranwa, J.N. and Kigondu, C.S. (2009). Reference Ranges of some Biochemical Parameters in Adult Kenyan. *International Journal of Health Research*; 2(3): 259-266.

Wakeman, L, Al-Ismail, S., Benton, A., Beddall, A., Gibbs, A., Hartnell, S., Morris,
 K. and Munro, R. (2007). Robust, routine haematology reference ranges for healthy adults. Internal Journal of Laboratory *Haematolology*, 29:279-283.

- Wang, H. (20116). Estimate of global, regional, and national incidence, prevalence and mortality of HIV, 1980 – 2015: The Global Burden of, Disease Study 2015. *Lancet HIV*, 3: e 361 – 87.
- Weber, J. (2001). The Pathogenesis of HIV 1 infection. *British Medical Bulletin*, 58(1): 61-72.
- Westerman, D.A., Evans, D. and Metz, J. (1999). Neutrophil hypersegmentation in iron deficiency anaemia: a case-control study. *British Journal Haematolology*, 107(3):512-515.
- Whitfield, J.B. (2001). Gamma-glutamyl transferase. *Critical Reviews in Clinical Laboratory Science*, 38: 263-355.
- Whitehead, T.P., Robinson, D. and Allaway, S.L. (1996). The effect of cigarette smoking and alcohol consumption on serum liver enzyme activities: a dose- related study in man. *Annals of Clinical Biochemistry*, 33: 530-535.
- WHO (2011). Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Vitamin and Mineral Nutrition Information System. Geneva. (WHO/NMH/NHD/MNM/11.1) (http://www.who.int/vmnis/indicators/haemoglobin
- Wide, L. and Bengtsson, C. (1990). Molecular charge heterogeneity of human serum erythropoietin. *British Journal of haematology*, **76**:121-127.

Winearls, C.G., Oliver, D.O., Pippard, M.J., Reid, C., Downing, M.R. and Cotes,
P.M. (1986). Effects of human erythropoietin derived from recombinant DNA on the anaemia of patients maintained by chronic haemodialysis. *Lancet*, 2(17): 1178.

- Wilson, E. M. and Sereti, I. (2013). Immune restoration after antiretroviral therapy: the pitfalls of hasty or incomplete repairs. *Immunology Reviews*. 254 (1): 343–354.
- Wolf, P.L. (1999). Biochemistry Diagnosis of liver disease. *Indian Journal of clinical biochemistry*, 14(1): 59 90.
- World Health organization, (2016). Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. *Recommendations* for a public health approach,  $2^{nd}$  Ed: 97 127.
- Yesuf, T., Muhie, O.A. and Shibru, H. (2019). Prevalence and predictors of anemia among adult HIV infected patients at the University of Gondar Hospital, Northwest Ethiopia. *Journals » HIV/AIDS - Research and Palliative Care 11:211-2.*
- Zeh, C.P.N., Inzaule, S. Ondoa, P. and Amornkul, P.N. (2011). Population-Based Biochemistry, Immunologic and Hematological Reference Values for Adolescents and Young Adults in a Rural Population in Western Kenya. *PLoS One.* 6(6): e21040.
- Zerihun, K.W., Bikis, G.A. and Muhammad, E.A (2019). Prevalence and associated Factors of anemia among adult human immune deficiency virus positive patients on anti-retroviral therapy at Debre tabor Hospital, Northwest Ethiopia. *BMC Research Notes*. 12: 168.https://doi.org/10.1186/s13104-019-4214-3
- Zizola, C. and Schulze, C.P. (2013). Metabolic and structural impairment of skeletal muscle in heart failure. *Heart Failure Reviews*. **18(5)**: 623–630.

#### **APPENDICES**

#### Appendix 1: Kenyatta National Hospital /University of Nairobi (KNH/UoN) Ethics

#### and Research Committee approval


## Appendix 1: continued

For more details consult the KNH/UoN -ERC website www.uonbi.ac.ke/activities/KNHUoN Kindly forward the informed consent documents for endorsement with updated stamp. Yours sincerely PROF. M.L. CHINDIA SECRETARY, KNH/UON-ERC The Principal, College of Health Sciences, UoN The Deputy Director, CS, KNH The Chairperson, KNH/UoN-ERC The Director, UNITID, UoN C.C. Protect to discover

#### Appendix 2: Consent participants 18- 60 years

#### **Project title**

Changes in serum erythropoietin, C-reactive protein, tumor necrosis factor-alpha, interleukin-6 levels in HIV-infected patients.

#### Introduction

My name is Jackson Ireri Mrama, a student at Kenyatta University carrying out the above research project in partial fulfillment of the requirements for the award of the degree of philosophy in immunohaematology. The objective of the study is to determine changes in serum levels of cytokines (serum proteins which effect biological changes in the body affecting changes in blood levels) that are novel markers for anaemia development and severity in HIV-infection to enable clinicians detect anaemia early for easier management.

#### Your role in the study

If you agree to participate in the study, you will be required to allow 5 militers of blood be taken from your arm.

#### **Study participants**

The study will involve a total of 300 HIV-positive people as cases and **240** HIVseronegative people as <u>referents</u>. Both ARV- treated and ARV-naïve cases will be recruited. All participants will be asked a few questions and some information obtained from their medical record files. There after five (5) militers (equivalent to one big tea spoon full) of blood will be taken from each participant for assaying the levels of the hormone (Epo), CD4+cell, CD8+ cells, TBC, CRP, U/E/C, LFTs, TNF- $\alpha$  and IL-6. The results of these tests will be used collectively to interpret the changes of the hormone levels in the blood. The information gathered from the result interpretations will help clinicians in detecting anaemia early before it manifests and effect management.

#### Benefits

There will be no monetary gains in participating in this study. However, all the tests will be done for <u>free in Paediatrics Laboratory of UoN.</u>

A five (5) milliliter blood sample will be taken from your arm. You may feel a little pain and discomfort for a short time. Rarely a small bruise may form at the site of injection but this goes away by itself after a few days.

#### Appendix 2: continued

#### Confidentiality

Your blood samples will not bear your names but study numbers. During presentation and/or publication of the test results, your names will not appear and there will be no way of anyone linking your identities with the results.

#### Freedom to withdraw

Participation in the study is purely voluntary. You have a right to decline or withdraw from the study at any time. If there are questions or issues you are uncomfortable to answer or discuss, you are not obliged to answer the questions or discuss the issues.

#### Declaration

I						. having been explained in my choice language
and	understood	about	this	study	by	
volu	ntarily give n	ny cons	ent to	partici	pate	in the study. I have been explained about my
right	s and assured	. I unde	rstand	l that ev	ven if	I refused to take part in the study I would lose
no m	edical benefi	ts.				

Signed	
Date	
Independent witness	
Name	Sign
Date	
Investigator' signature	Date

**Appendix 3: Quality Control for CD4 Cell Counts** 

(i) CD4 Commercial control



Low CD4 Abs Control					
Lot #	ABC123				
+3 SD	210				
+2 SD	190				
+1 SD	170				
Mean	150				
-1 SD	130				
-2 SD	110				
-3 SD	90				

Normal CD4 Abs Control					
Lot #	ACB123				
+3 SD	1000				
+2 SD	900				
+1 SD	800				
Mean	700				
-1 SD	600				
-2 SD	500				
-3SD	400				

# Appedix 4: Quality Control Haematological Parameters

Parameter	Expected low limit	Control result	Expected High Limit	Remarks
HB g/dl	12.8	13.0	14.2	Acceptable
RBC x 10 <sup>12</sup>	4.4	4.1	4.8	Acceptable
MCV Fl	78	82.5	87	Acceptable
MCH pg/dl	28.3	31.7	32.3	Acceptable
MCHC g/dl	32.4	38.4	38.4	Acceptable
WBC x 10 <sup>9</sup> /l	6.0	6.89	8.4	Acceptable
N x 10 <sup>9</sup> /l	3.36	4.1	5.88	Acceptable
L x 10 <sup>9</sup> /l	1.55	2.6	3.19	Acceptable
M x 10 <sup>9</sup> /l	1.0	2.1	9.0	Acceptable
E x 10 <sup>9</sup> /l	0.24	0.3	0.67	Acceptable
PLTs x 10 <sup>9</sup> /l	155	212	245	Acceptable

Bouchon ® Commercial control for Haematological Parameters

# **Appendix 5: Quality Control for Biochemical parameters**

Parameter	Expected	Control	Expected	Remarks
	low limit	result	High Limit	
AST u/l	27	37	43	Acceptable
ALT u/l	27	39	43	Acceptable
GGT u/l	27	38	42	Acceptable
ALP u/l	134	185	223	Acceptable
T.PRT g/l	57.30	65.23	71.50	Acceptable
ALB g/l	26.40	35.61	42.20	Acceptable
CRT mmoles //l	79.50	116.73	120.0	Acceptable
UREA µmoles /l	4.40	4.90	6.80	Acceptable
T. Bill µmoles /l	21.80	24.71	37.10	Acceptable
D. Bill µmoles /l	21.10	25.54	34.30	Acceptable

# SERODOS 004 ® Commercial Control for Biochemistry values

	<b>T</b> 7 •	<b>.</b> .		<b>a</b> • •	<b>T</b> 7•11 4 4	
Parameter	Koimongr	ov-Smi	rova	Shapiro-V	Vilk test	
	Statistic	Df	Sig.	Statistic	Df	Sig.
HB	0.091	107	0.029	0.929	107	< 0.001
RBC	0.082	107	0.068	0.93	107	< 0.001
MCV	0.132	107	< 0.001	0.922	107	< 0.001
MCH	0.102	107	< 0.001	0.96	107	0.003
MCHC	0.199	107	< 0.001	0.564	107	< 0.001
WBC	0.118	107	0.001	0.96	107	0.003
Ν	0.103	107	0.007	0.943	107	< 0.001
L	0.095	107	0.02	0.98	107	0.115
М	0.194	107	< 0.001	0.883	107	< 0.001
E	0.369	107	0.001	0.553	107	< 0.001
Р	0.118	107	0.001	0.917	107	< 0.001

## Appendix 6: Tests the Normality of Referents' Parameters

**Referent female haematological parameters** 

(i)

Shapiro – Wilk test, P > 0.05 is significant

## (ii) Referent male haematological parameters

Doromotor	Koimong	rov-Smir	ova	Shapiro-		
	Statistic	Df	Sig.	Statistic	Df	Sig.
HB	0.132	93	< 0.001	0.926	93	< 0.001
RBC	0.094	93	0.043	0.936	93	< 0.001
MCV	0.098	93	0.028	0.976	93	0.09
MCH	0.083	93	0.134	0.961	93	0.008
MCHC	0.094	93	0.345	0.895	93	< 0.001
WBC	0.093	93	0.2	0.984	93	0.3
Ν	0.123	93	0.001	0.959	93	0.005
L	0.084	93	0.114	0.985	93	0.341
М	0.221	93	< 0.001	0.855	93	0.001
Е	0.374	93	< 0.001	0.659	93	< 0.001
Р	0.088	93	0.069	0.927	93	< 0.001

Shapiro – Wilk test, P > 0.05 is significant

Doromotor	Koimong	grov-Sm	irova	Shapiro-Wilk test		
I al allietel	Statistic	Df	Sig.	Statistic	Df	Sig.
AST	0.09	107	0.032	0.973	107	0.027
ALT	0.118	107	0.001	0.917	107	0
GGT	0.168	107	0	0.703	107	0
ALP	0.077	107	0.141	0.981	107	0.137
T.Bil	0.136	107	0	0.897	107	0
D.Bil	0.144	107	0	0.909	107	0
T.PRT	0.062	107	.200*	0.985	107	0.271
ALB	0.128	107	0	0.946	107	0
UREA	0.162	107	0	0.867	107	0
CRT	0.157	107	0	0.879	107	0

## (iv) Referent female Biochemistry parameters

Shapiro – Wilk test, P > 0.05 is significant

## (v) Referent males Biochemistry parameters

	Koimong	rov-Smir	ova	Shapiro-Wilk test		
Parameter	Statistic	Df	Sig.	Statistic	Df	Sig.
AST	0.179	93	<.0.001	0.776	93	< 0.001
ALT	0.202	93	< 0.001	0.747	93	< 0.001
GGT	0.202	93	< 0.001	0.64	93	< 0.001
ALP	0.098	93	0.028	0.938	93	< 0.001
T.Bil	0.135	93	< 0.001	0.837	93	< 0.001
D.Bil	0.164	93	< 0.001	0.814	93	< 0.001
T.PRT	0.068	93	.200	0.985	93	0.351
ALB	0.084	93	0.114	0.983	93	0.267
UREA	0.182	93	< 0.001	0.772	93	< 0.001
CRT	0.146	93	< 0.001	0.807	93	< 0.001

		Kolmogorov-Smirnov			Sha	apiro-W	ïlk
Parameter	Gender	Statistic	df	Sig.	Statistic	df	Sig.
EPO0	Female	0.359	107	0.000	0.360	107	< 0.001
	Male	0.281	93	0.000	0.784	93	< 0.001
TNF0	Female	0.409	107	0.000	0.225	107	< 0.001
	Male	0.410	93	0.000	0.205	93	< 0.001
IL60	Female	0.413	107	0.000	0.182	107	< 0.001
	Male	0.398	93	0.000	0.221	93	< 0.001
CRP0	Female	0.140	107	0.000	0.900	107	< 0.001
	Male	0.184	93	0.000	0.821	93	< 0.001

# (vi) Referent female and Epo, TNF-, IL-6 and CRP Parameters

Shapiro – Wilk test, P > 0.05 is significant

## Appendix 7: Tests for the Normality of HIV Positive Respondents' Parameters

	Koimongrov-Smirova			Shapiro-Wilk test			
Parameter	Statistics	Df	Sig.	Statistics	Df	Sig.	
RBC F <sub>0</sub>	0.057	95	.200	0.991	95	0.775	
<b>RBC F</b> <sub>1</sub>	0.07	95	.200	0.982	95	0.214	
RBC F <sub>2</sub>	0.084	95	0.094	0.974	95	0.058	
HB Fo	0.077	95	.200	0.99	95	0.725	
HB F <sub>1</sub>	0.093	95	0.042	0.974	95	0.060	
HB F <sub>2</sub>	0.129	95	< 0.001	0.813	95	< 0.001	
MCV F <sub>0</sub>	0.08	95	0.154	0.981	95	0.173	
MCV F <sub>1</sub>	0.089	95	0.060	0.97	95	0.026	
MCV F <sub>2</sub>	0.099	95	0.022	0.97	95	0.029	
MCH F <sub>0</sub>	0.084	95	0.097	0.972	95	0.037	
MCH F <sub>1</sub>	0.098	95	0.025	0.95	95	0.001	
MCH F <sub>2</sub>	0.089	95	0.060	0.976	95	0.075	
MCHC F <sub>0</sub>	0.152	95	< 0.001	0.81	95	< 0.001	
MCHC F <sub>1</sub>	0.215	95	< 0.001	0.707	95	< 0.001	
MCHC F <sub>2</sub>	0.12	95	0.002	0.964	95	0.011	
WBC F <sub>0</sub>	0.098	95	0.025	0.922	95	< 0.001	
WBC F <sub>1</sub>	0.08	95	0.161	0.943	95	< 0.001	
WBC F <sub>2</sub>	0.136	95	< 0.001	0.941	95	< 0.001	
N F <sub>0</sub>	0.134	95	< 0.001	0.929	95	< 0.001	
$\mathbf{N} \mathbf{F}_1$	0.109	95	0.007	0.946	95	< 0.001	
NF <sub>2</sub>	0.13	95	< 0.001	0.928	95	< 0.001	
	0.116	95 05	0.003	0.964	95 05	0.011	
	0.095	95 05	0.034	0.906	95 05	< 0.001	
	0.16/	95 05	< 0.001	0.936	95 05	< 0.001	
MF.	0.138	9 <i>5</i> 05	< 0.001	0.928	95 05	< 0.001	
	0.175	95	< 0.001	0.313	95	< 0.001	
E FO	0.303	95	< 0.001	0.313	95	< 0.001	
	0.274	95	< 0.001	0.549	95	< 0.001	
E Fa	0.269	95	< 0.001	0.711	95	< 0.001	
	0.058	95	200	0.99	95	0.732	
PF1	0.092	95 95	0.044	0.892	95	< 0.001	
PF1	0.106	95	0.011	0.962	95	0.008	

## (i) ART female haematological parameters

Shapiro – Wilk test, P > 0.05 is significant  $F_0$ =Baseline,  $F_1 = 3$  months,  $F_2 = 6$  months

	Kolmogorov-Smirnov			Shapiro-Wilk			
Parameter	Gender	Statistic	df	Sig.	Statistic	df	Sig.
MCHC F <sub>2</sub>	Male	0.084	55	.200	0.985	55	0.734
WBC F <sub>0</sub>	Male	0.135	55	0.014	0.929	55	0.003
WBC F <sub>1</sub>	Male	0.065	55	$.200^{*}$	0.974	55	0.289
WBC F <sub>2</sub>	Male	0.091	55	$.200^{*}$	0.979	55	0.432
N F <sub>0</sub>	Male	0.143	55	0.007	0.868	55	< 0.001
N F <sub>1</sub>	Male	0.13	55	0.022	0.969	55	0.16
N F <sub>2</sub>	Male	0.127	55	0.027	0.966	55	0.122
L F <sub>0</sub>	Male	0.143	55	0.007	0.912	55	0.001
$L F_1$	Male	0.097	55	.200	0.985	55	0.704
$L F_2$	Male	0.113	55	0.08	0.947	55	0.017
$M F_0$	Male	0.285	55	< 0.001	0.81	55	< 0.001
$M F_2$	Male	0.201	55	.<0001	0.745	55	< 0.001
E F0	Male	0.255	55	< 0,001	0.494	55	< 0.001
$E F_1$	Male	0.284	55	< 0.001	0.647	55	< 0.001
$E F_2$	Male	0.335	55	< 0.001	0.378	55	< 0.001
$P F_0$	Male	0.097	55	.200	0.984	55	0.693
$P F_1$	Male	0.066	55	.200	0.969	55	0.166
$PF_2$	Male	0.07	55	.200	0.987	55	0.805

## (ii) **ART male haematological parameters**

Parameter	Gender	Kolmogor	ov-Sm	irnov	Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
HB F <sub>0</sub>	Female	0.176	22	0.074	0.896	22	0.024
HB F <sub>1</sub>	Female	0.201	22	0.021	0.904	22	0.036
HB F <sub>2</sub>	Female	0.157	22	0.166	0.903	22	0.035
RBC F <sub>0</sub>	Female	0.121	22	.200	0.933	22	0.141
RBC F <sub>1</sub>	Female	0.162	22	0.140	0.947	22	0.276
MCV F <sub>0</sub>	Female	0.113	22	.200	0.966	22	0.610
MCV F <sub>1</sub>	Female	0.157	22	0.172	0.967	22	0.638
MCV F <sub>2</sub>	Female	0.163	22	0.134	0.959	22	0.463
MCH F <sub>0</sub>	Female	0.096	22	.200	0.956	22	0.407
MCH F <sub>1</sub>	Female	0.211	22	0.012	0.958	22	0.448
MCH F2	Female	0.158	22	0.163	0.938	22	0.181
MCHC f <sub>0</sub>	Female	0.110	22	.200	0.964	22	0.584
MCHC F1	Female	0.165	22	0.124	0.930	22	0.121
MCHC F <sub>2</sub>	Female	0.217	22	0.008	0.640	22	0.000
WBC F <sub>0</sub>	Female	0.273	22	0.000	0.702	22	0.000
WB C F <sub>1</sub>	Female	0.087	22	.200	0.968	22	0.663
WBC $f_2$	Female	0.136	22	.200	0.955	22	0.388
$N F_0$	Female	0.312	22	0.000	0.532	22	0.000
$N F_1$	Female	0.161	22	0.141	0.922	22	0.083
N F <sub>2</sub>	Female	0.113	22	.200	0.970	22	0.720
L F <sub>0</sub>	Female	0.135	22	.200	0.906	22	0.039
$L F_1$	Female	0.203	22	0.018	0.925	22	0.096
L F <sub>2</sub>	Female	0.229	22	0.004	0.882	22	0.013
$M F_0$	Female	0.287	22	0.000	0.773	22	0.000
M F1	Female	0.220	22	0.007	0.907	22	0.040
$M F_2$	Female	0.204	22	0.018	0.894	22	0.023
$E F_0$	Female	0.253	22	0.001	0.695	22	0.000
$E F_0$	Female	0.231	22	0.004	0.766	22	0.000
EF <sub>2</sub>	Female	0.291	22	0.000	0.715	22	0.000
$\mathbf{P} \mathbf{F}_0$	Female	0.222	22	0.006	0.743	22	0.000
$\mathbf{P} \mathbf{F}_1$	Female	0.216	22	0.009	0.817	22	0.001
$P F_2$	Female	0.143	22	.200	0.894	22	0.022

## (iii) ARV- naive female haematological parameters

$$\label{eq:F0} \begin{split} F_0 &= Baseline, \, F_1 = 3 \mbox{ months}, \, F_2 = 6 \mbox{ months} \\ Shapiro - Wilk \mbox{ test}, \, P > 0.05 \mbox{ is significant} \end{split}$$

	liatological	paran	ietei s	
_	Kolmoge	orov-Sn	nirnov	Sha
Gender	Statistic	df	Sig.	Statistic
Male	0.229	7	.200	0.908
Male	0.203	7	.200	0.913
Male	0.259	7	0.172	0.812
Male	0.332	7	0.019	0.869

Shapiro-Wilk

#### ARV- naive male haematological parameters (iii)

Parameter	Gender	Statistic	df	Sig.	Statistic	df	Sig.
HB $F_0$	Male	0.229	7	.200	0.908	7	0.385
HB $F_1$	Male	0.203	7	.200	0.913	7	0.416
HB F <sub>2</sub>	Male	0.259	7	0.172	0.812	7	0.054
$RBC F_0$	Male	0.332	7	0.019	0.869	7	0.183
RBC F <sub>1</sub>	Male	0.166	7	.200	0.983	7	0.971
RBC F <sub>2</sub>	Male	0.186	7	.200	0.916	7	0.442
$MCV F_0$	Male	0.161	7	.200	0.954	7	0.762
MCV F <sub>1</sub>	Male	0.172	7	.200	0.974	7	0.925
MCV F <sub>2</sub>	Male	0.163	7	.200	0.952	7	0.748
MCH F <sub>0</sub>	Male	0.218	7	.200	0.947	7	0.704
MCH F <sub>1</sub>	Male	0.219	7	.200	0.903	7	0.352
MCH F <sub>2</sub>	Male	0.133	7	.200	0.973	7	0.919
MCHC F <sub>0</sub>	Male	0.292	7	0.072	0.784	7	0.028
MCHC F <sub>1</sub>	Male	0.300	7	0.057	0.877	7	0.214
MCHC F <sub>2</sub>	Male	0.147	7	.200	0.960	7	0.817
WBC $F_0$	Male	0.335	7	0.017	0.803	7	0.044
WBC F <sub>1</sub>	Male	0.289	7	0.080	0.889	7	0.267
WBC F <sub>2</sub>	Male	0.229	7	.200	0.904	7	0.358
$N F_0$	Male	0.270	7	0.131	0.825	7	0.071
$N F_1$	Male	0.187	7	.200	0.902	7	0.340
$N F_2$	Male	0.357	7	0.007	0.758	7	0.015
L F <sub>0</sub>	Male	0.178	7	$.200^{*}$	0.949	7	0.723
$L F_1$	Male	0.177	7	.200	0.965	7	0.861
$L F_2$	Male	0.170	7	.200	0.956	7	0.783
$\mathbf{M} \mathbf{F}_0$	Male	0.318	7	0.031	0.671	7	0.002
$\mathbf{M} \mathbf{F}_1$	Male	0.173	7	$.200^{*}$	0.922	7	0.482
$M F_2$	Male	0.172	7	.200	0.967	7	0.873
E F <sub>0</sub>	Male	0.389	7	0.002	0.672	7	0.002
$E F_1$	Male	0.338	7	0.015	0.822	7	0.067
$E F_2$	Male	0.240	7	.200	0.864	7	0.163
PF <sub>0</sub>	Male	0.206	7	.200	0.941	7	0.651
$\mathbf{P} \mathbf{F}_1$	Male	0.171	7	.200	0.972	7	0.916
P F <sub>2</sub>	Male	0.169	7	.200	0.975	7	0.929

		Kolmogo	Kolmogorov-Smirnov			Shapiro-Wilk		
Parameter	Gender	Statistic	df	Sig.	Statistic	df	Sig.	
AST F <sub>0</sub>	Female	0.200	95	< 0.001	0.840	95	< 0.001	
AST F1	Female	0.124	95	0.001	0.883	95	< 0.001	
AST F <sub>2</sub>	Female	0.281	95	< 0.001	0.334	95	< 0.001	
ALT F <sub>0</sub>	Female	0.126	95	0.001	0.854	95	< 0.001	
ALT F <sub>1</sub>	Female	0.150	95	< 0.001	0.827	95	< 0.001	
ALT F <sub>2</sub>	Female	0.166	95	< 0.001	0.675	95	< 0.001	
G-GT F <sub>0</sub>	Female	0.187	95	< 0.001	0.763	95	< 0.001	
G-GT F1	Female	0.177	95	< 0.001	0.832	95	< 0.001	
G-GT F <sub>2</sub>	Female	0.160	95	< 0.001	0.794	95	< 0.001	
ALP F <sub>0</sub>	Female	0.158	95	< 0.001	0.892	95	< 0.001	
ALP F <sub>1</sub>	Female	0.146	95	< 0.001	0.857	95	< 0.001	
ALP F <sub>2</sub>	Female	0.056	95	.200	0.991	95	0.742	
T.BIL F <sub>0</sub>	Female	0.179	95	< 0.001	0.847	95	< 0.001	
T.BIL $F_1$	Female	0.075	95	.200	0.940	95	< 0.001	
T.BIL F <sub>2</sub>	Female	0.181	95	< 0.001	0.825	95	< 0.001	
$D.BILF_0$	Female	0.273	95	< 0.001	0.785	95	< 0.001	
D.BIL $F_1$	Female	0.226	95	< 0.001	0.787	95	< 0.001	
D.BIL F <sub>2</sub>	Female	0.255	95	< 0.001	0.711	95	< 0.001	
T.PRT $F_0$	Female	0.057	95	$.200^{*}$	0.992	95	< 0.001	
T.PRT $F_1$	Female	0.106	95	0.010	0.930	95	< 0.001	
T.PRT F <sub>2</sub>	Female	0.104	95	0.013	0.979	95	0.130	
ALB F <sub>0</sub>	Female	0.134	95	< 0.001	0.943	95	< 0.001	
ALB F <sub>1</sub>	Female	0.113	95	0.005	0.972	95	0.040	
ALB F <sub>2</sub>	Female	0.092	95	0.047	0.986	95	0.415	
UREA F <sub>0</sub>	Female	0.098	95	0.026	0.962	95	0.008	
UREA F1	Female	0.154	95	< 0.001	0.767	95	< 0.001	
UREA F <sub>2</sub>	Female	0.129	95	0.001	0.837	95	< 0.001	
CRT F <sub>0</sub>	Female	0.154	95	< 0.001	0.842	95	< 0.001	
CRT F <sub>1</sub>	Female	0.142	95	< 0.001	0.807	95	< 0.001	
CRT F <sub>2</sub>	Female	0.124	95	0.001	0.915	95	< 0.001	

## (iv) ART female Biochemical parameters

Shapiro – Wilk test, P > 0.05 is significant

 $F_0$ =Baseline,  $F_1 = 3$  months,  $F_2 = 6$  months

	_	Kolmogorov-Smirnov			Shapiro-Wilk		
Parameter G	ender	Statistic	df	Sig.	Statistic	df	Sig.
AST F <sub>0</sub>	Male	0.179	55	< 0.001	0.898	55	< 0.001
AST F <sub>1</sub>	Male	0.207	55	< 0.001	0.795	55	< 0.001
AST F <sub>2</sub>	Male	0.167	55	0.001	0.804	55	< 0.001
ALT F <sub>0</sub>	Male	0.163	55	0.001	0.893	55	< 0.001
ALT F <sub>1</sub>	Male	0.106	55	0.190	0.921	55	0.001
ALT F <sub>2</sub>	Male	0.158	55	0.002	0.816	55	< 0.001
G-GT F <sub>0</sub>	Male	0.349	55	< 0.001	0.528	55	< 0.001
G-GT F <sub>1</sub>	Male	0.276	55	< 0.001	0.652	55	< 0.001
G-GT F <sub>2</sub>	Male	0.197	55	< 0.001	0.721	55	< 0.001
ALP F <sub>0</sub>	Male	0.256	55	< 0.001	0.449	55	< 0.001
ALP F <sub>1</sub>	Male	0.130	55	0.022	0.936	55	0.006
ALP F <sub>2</sub>	Male	0.142	55	0.008	0.944	55	0.012
T.BIL F <sub>0</sub>	Male	0.183	55	0.000	0.737	55	< 0.001
$T.BIL F_1$	Male	0.128	55	0.026	0.925	55	0.002
T.BIL F <sub>2</sub>	Male	0.131	55	0.019	0.942	55	0.011
D.BIL F <sub>0</sub>	Male	0.218	55	< 0.001	0.736	55	< 0.001
$D.BILF_1$	Male	0.187	55	< 0.001	0.826	55	< 0.001
D.BIL F <sub>2</sub>	Male	0.292	55	< 0.001	0.670	55	< 0.001
$T.PRT F_0$	Male	0.081	55	.200	0.964	55	0.096
$T.PRT F_1$	Male	0.146	55	0.005	0.944	55	0.012
T.PRT F <sub>2</sub>	Male	0.089	55	.200	0.975	55	0.319
ALB F <sub>0</sub>	Male	0.098	55	.200	0.979	55	0.448
ALB F <sub>1</sub>	Male	0.105	55	0.198	0.980	55	0.501
ALB F <sub>2</sub>	Male	0.129	55	0.024	0.914	55	0.001
UREA F <sub>0</sub>	Male	0.206	55	< 0.001	0.736	55	< 0.001
UREA F1	Male	0.063	55	.200	0.984	55	0.680
UREA F <sub>2</sub>	Male	0.169	55	< 0.001	0.918	55	0.001
CRT F <sub>0</sub>	Male	0.185	55	< 0.001	0.635	55	< 0.001
CRT F <sub>1</sub>	Male	0.317	55	< 0.001	0.308	55	< 0.001
CRT F <sub>2</sub>	Male	0.074	55	.200	0.976	55	0.328

## (V) ART male Biochemical parameters

		Kolmogorov-Smirnov			Shapiro-Wilk		
Parameter	Gender	Statistic	df	Sig.	Statistic	df	Sig.
AST F <sub>0</sub>	Female	0.194	22	0.031	0.895	22	0.023
AST F <sub>1</sub>	Female	0.155	22	0.180	0.931	22	0.130
AST F <sub>2</sub>	Female	0.135	22	.200	0.926	22	0.101
ALT F <sub>0</sub>	Female	0.129	22	.200	0.939	22	0.186
ALT F <sub>1</sub>	Female	0.183	22	0.053	0.845	22	0.003
ALT F <sub>2</sub>	Female	0.140	22	.200	0.961	22	0.518
G-GT F <sub>0</sub>	Female	0.210	22	0.013	0.828	22	< 0.001
G-GT F <sub>1</sub>	Female	0.261	22	< 0.001	0.714	22	< 0.001
G-GT F <sub>2</sub>	Female	0.239	22	0.002	0.641	22	< 0.001
ALP F <sub>0</sub>	Female	0.102	22	.200	0.959	22	0.467
ALP F <sub>1</sub>	Female	0.166	22	0.115	0.908	22	0.044
ALP F <sub>2</sub>	Female	0.104	22	.200	0.970	22	0.700
T.BIL F <sub>0</sub>	Female	0.193	22	0.033	0.867	22	0.007
T.BIL $F_1$	Female	0.246	22	0.001	0.837	22	0.002
T.BIL F <sub>2</sub>	Female	0.159	22	0.153	0.878	22	0.011
D.BIL F <sub>0</sub>	Female	0.207	22	0.015	0.812	22	0.001
$D.BILF_1$	Female	0.277	22	< 0.001	0.659	22	< 0.001
D.BILF <sub>2</sub>	Female	0.274	22	< 0.001	0.668	22	< 0.001
T.PRT F <sub>0</sub>	Female	0.149	22	.200	0.972	22	0.762
T.PRT $F_1$	Female	0.152	22	.200	0.961	22	0.502
T.PRT F <sub>2</sub>	Female	0.140	22	.200	0.941	22	0.203
ALB F <sub>0</sub>	Female	0.156	22	0.178	0.922	22	0.084
ALB F <sub>1</sub>	Female	0.165	22	0.122	0.898	22	0.028
ALB F <sub>2</sub>	Female	0.119	22	.200	0.969	22	0.696
UREA F <sub>0</sub>	Female	0.155	22	0.182	0.939	22	0.193
UREA F1	Female	0.166	22	0.117	0.936	22	0.164
UREA F <sub>2</sub>	Female	0.539	22	< 0.001	0.222	22	0.000
CRT F <sub>0</sub>	Female	0.114	22	.200	0.972	22	0.746
CRT F1	Female	0.102	22	.200	0.943	22	0.233
CRT F <sub>2</sub>	Female	0.087	22	.200	0.966	22	0.611

# (iv) ARV-naive female Biochemical parameters

# (v) ARV-naive male Biochemical parameters

		Kolmogorov-Smirnov			Shapiro-Wilk		
Parameter G	lender	Statistic	df	Sig.	Statistic	df	Sig.
AST F <sub>0</sub>	Male	0.329	7	0.021	0.858	7	0.145
AST $F_1$	Male	0.140	7	.200	0.973	7	0.922
AST F <sub>2</sub>	Male	0.271	7	0.130	0.853	7	0.131
ALT F <sub>0</sub>	Male	0.185	7	.200	0.967	7	0.874
ALT F <sub>1</sub>	Male	0.250	7	.200	0.885	7	0.248
ALT F <sub>2</sub>	Male	0.201	7	.200	0.939	7	0.630
G-GT F <sub>0</sub>	Male	0.219	7	.200	0.935	7	0.592
G-GT F <sub>1</sub>	Male	0.205	7	.200	0.899	7	0.325
G-GT F <sub>2</sub>	Male	0.271	7	0.130	0.866	7	0.172
ALP F <sub>0</sub>	Male	0.177	7	$.200^{*}$	0.940	7	0.641
ALP F <sub>1</sub>	Male	0.268	7	0.138	0.914	7	0.423
ALP F <sub>2</sub>	Male	0.250	7	.200	0.825	7	0.071
T.BI L F <sub>0</sub>	Male	0.280	7	0.102	0.874	7	0.201
T.BIL F1	Male	0.158	7	.200	0.948	7	0.713
T.BIL F <sub>2</sub>	Male	0.307	7	0.046	0.830	7	0.081
D.BIL F <sub>0</sub>	Male	0.351	7	0.009	0.715	7	0.005
D.BIL F <sub>1</sub>	Male	0.284	7	0.093	0.802	7	0.043
D.BIL F <sub>2</sub>	Male	0.434	7	< 0.001	0.630	7	0.001
T.PRT F <sub>0</sub>	Male	0.220	7	.200	0.952	7	0.744
T.PRT $F_1$	Male	0.189	7	.200	0.938	7	0.624
T.PRT F <sub>2</sub>	Male	0.188	7	.200	0.947	7	0.700
ALB F <sub>0</sub>	Male	0.295	7	0.066	0.885	7	0.249
ALB F <sub>1</sub>	Male	0.292	7	0.072	0.889	7	0.269
ALB F <sub>2</sub>	Male	0.275	7	0.117	0.758	7	0.015
UREA F <sub>0</sub>	Male	0.292	7	0.073	0.868	7	0.178
UREA F <sub>1</sub>	Male	0.197	7	.200	0.947	7	0.704
UREA F <sub>2</sub>	Male	0.228	7	.200	0.897	7	0.313
CRT F <sub>0</sub>	Male	0.194	7	.200	0.918	7	0.451
CRT F1	Male	0.240	7	.200	0.897	7	0.312
CRT F <sub>2</sub>	Male	0.217	7	.200	0.873	7	0.199

	_	Kolmogorov-Smirnov			Shapiro-Wilk		
Parameter	Gender	Statistic	df	Sig.	Statistic	df	Sig.
EPO F <sub>0</sub>	Female	0.156	22	0.178	0.874	22	0.009
	Male	0.413	7	0.001	0.581	7	< 0.001
EPO F <sub>1</sub>	Female	0.102	22	.200	0.951	22	0.332
	Male	0.199	7	.200	0.869	7	0.183
EPO F <sub>2</sub>	Female	0.191	22	0.036	0.880	22	0.012
	Male	0.293	7	0.069	0.831	7	0.082
TNF- $\alpha$ F <sub>0</sub>	Female	0.113	22	.200	0.953	22	0.364
	Male	0.165	7	.200	0.893	7	0.291
TNF- $\alpha$ F <sub>1</sub>	Female	0.190	22	0.037	0.828	22	0.001
	Male	0.181	7	$.200^{*}$	0.958	7	0.805
TNF- $\alpha$ F <sub>2</sub>	Female	0.146	22	.200	0.947	22	0.272
	Male	0.214	7	.200	0.869	7	0.184
IL6 F <sub>0</sub>	Female	0.313	22	0.000	0.663	22	< 0.001
	Male	0.396	7	0.001	0.588	7	< 0.001
IL6 $F_1$	Female	0.280	22	< 0.001	0.701	22	< 0.001
	Male	0.294	7	0.069	0.817	7	0.061
IL6 F <sub>2</sub>	Female	0.434	22	< 0.001	0.603	22	0.000
	Male	-	7	-	-	7	-
CRP F <sub>0</sub>	Female	0.436	22	< 0.001	0.616	22	< 0.001
	Male	0.177	7	.200	0.890	7	0.276
CRP F1	Female	0.220	22	0.007	0.837	22	0.002
	Male	0.162	7	.200	0.965	7	0.862
CRP F <sub>2</sub>	Female	0.257	22	0.001	0.689	22	< 0.001
	Male	0.335	7	0.017	0.778	7	0.024

# (vi) ARV-naive female and male Epo, TNF, IL-6 and CRP parameters

$$\label{eq:F0} \begin{split} F_0 &= Baseline, \ F_1 = 3 \ months, \ F_2 = 6 \ months \\ Shapiro - Wilk \ test, \ P > 0.05 \ is \ significant \end{split}$$

		Kolmogorov-Smirnov		Shapiro-Wilk				
					Statist			
Parameter C	Gender	Statistic	df	Sig.	ic	df	Sig.	
EPO F <sub>0</sub>	Female	0.201	95	< 0.001	0.641	95	< 0.001	
	Male	0.328	55	< 0.001	0.427	55	< 0.001	
EPO F <sub>1</sub>	Female	0.148	95	< 0.001	0.912	95	< 0.001	
	Male	0.143	55	0.007	0.903	55	< 0.001	
EPO F <sub>2</sub>	Female	0.140	95	< 0.001	0.844	95	< 0.001	
	Male	0.146	55	0.005	0.847	55	< 0.001	
TNF- $\alpha$ F <sub>0</sub>	Female	0.131	95	< 0.001	0.874	95	< 0.001	
	Male	0.134	55	0.015	0.922	55	0.002	
TNF- $\alpha$ F <sub>1</sub>	Female	0.317	95	< 0.001	0.451	95	< 0.001	
	Male	0.221	55	< 0.001	0.780	55	< 0.001	
TNF- $\alpha$ F <sub>2</sub>	Female	0.146	95	< 0.001	0.869	95	< 0.001	
	Male	0.116	55	0.062	0.935	55	0.005	
IL6 F <sub>0</sub>	Female	0.340	95	< 0.001	0.458	95	< 0.001	
	Male	0.289	55	< 0.001	0.623	55	< 0.001	
IL6 F <sub>1</sub>	Female	0.279	95	< 0.001	0.638	95	< 0.001	
	Male	0.281	55	< 0.001	0.643	55	< 0.001	
IL6 F <sub>2</sub>	Female	0.386	95	< 0.001	0.606	95	< 0.001	
	Male	0.417	55	< 0.001	0.513	55	< 0.001	
CRP F <sub>0</sub>	Female	0.246	95	< 0.001	0.656	95	< 0.001	
	Male	0.139	55	0.010	0.868	55	< 0.001	
CRP F <sub>1</sub>	Female	0.185	95	< 0.001	0.819	95	< 0.001	
	Male	0.174	55	< 0.001	0.796	55	< 0.001	
CRP F <sub>2</sub>	Female	0.206	95	< 0.001	0.726	95	< 0.001	
	Male	0.324	55	< 0.001	0.374	55	< 0.001	

# (vii) ARV-naive female and male Epo, TNF, IL-6 and CRP parameters