Effect of Antiretroviral Regimens on Haematological and Immunological Abnormalities of People Living With Hiv in a Comprehensive Care Centre in Kiambu County, Kenya

Esther Wangui Mandania[1*], Stanley Kinge Waithaka[1], Suliman Essuman[2]

1.Department of Medical Laboratory Sciences, Medical School, Mount Kenya University, P.O. Box 342-01000, Thika, Kenya
2. Department of Microbiology, Medical School, Mount Kenya University, P.O. Box 342-01000, Thika, Kenya

Abstract

Background: Haematological abnormalities are linked to a higher risk of HIV disease progression and mortality. Quality of life is enhanced via the restoration and maintenance of immune function through the efficient administration of antiretroviral treatment (ART). Nevertheless, ART has the potential to favorably or adversely affect haematological parameters. The purpose of this study was to compare the effects of various ART regimens on immunohaematological parameters abnormalities in adult patients living with HIV.

Methods: This cross-sectional study enrolled 237 participants, between July 2022 to December 2023 at Thika Level Five Hospital's Comprehensive Care Centre (CCC) in Kiambu County, Kenya. Total blood count and the CD4+ T cells count were measured using standard laboratory procedures. Sociodemographic data, clinical characteristics and type of ART regimens were collected by use of a structured questionnaire and review of patient medical records.

Results: The mean levels of PLT, ALC, HB, MCV, MCH, PCV, of ART – treated was significantly higher compared with those of ART – naïve ($p < 0.05$). However, the mean levels of CD4 count showed no statistically significant difference between ART-treated and ART-naïve individuals ($p=0.5045$). TDF/3TC/DTG regimen was associated with higher lymphopenia rates ((50.00 %, $p = 0.0252$) while AZT/3TC/ATV/r was associated with high rates of macrocytosis (35.29%, $p<0.0001$). Hypochromia was significantly higher in those on TDF/3TC/DTG (94.12%, $p=0.0268$). Elevated RDW was more frequent in patients on TDF/3TC/DTG (60.00%, $p=0.0044$).

Conclusion: PLT, ALC, HB, MCV, MCH, PCV, of ART – treated were significantly higher compared with those of ART – naïve. TDF/3TC/DTG regimen significantly influenced lymphopenia while AZT/3TC/ATV/r was associated with high rates of macrocytosis. Regular monitoring and treatment of HIV- infected patients on ART for haematological and immunological abnormalities is vital in order to reduce HIV- related morbidities. Clinicians should consider ART –associated adverse effects when selecting ART regimens.

Key words: Haematological abnormalities; ART regimen, ART- treated, Kenya

* Corresponding author: emandania@yahoo.com

Introduction

Human immunodeficiency virus (HIV), a lentivirus belonging to the retroviridae family, is the causative agent of the HIV/AIDS pandemic [(1)]. One of the most pressing issues in public health in the last few decades has been the worldwide epidemic of HIV/AIDS [(2)]. The illness has had a profound effect on people, communities, and economies throughout Sub-Saharan Africa (SSA) [(3)].

According to the Joint United Nations Program on HIV/AIDS (UNAIDS), around 39 million individuals throughout the world were living with HIV as of 2022. About 1.3 million people were infected with HIV that year, and 630,000 died from AIDS-related causes. At least 40.4 million individuals have lost their lives to AIDS-related causes since the virus first appeared. The number of fatalities caused by HIV/AIDS decreased by 69% from its peak in 2004 to 2022, when more than 29 million individuals living with the virus were getting antiretroviral therapy. In 2022, there were 630,000 deaths worldwide due to HIV, with 3 out of 5 of those fatalities occurring in Africa, despite the fact that access to antiretroviral therapy (ART) has improved. almost 25.6 million people, or almost two-thirds of the world's HIV diagnoses, live in Sub-Saharan Africa, making it the worst-hit area. About 1.4 million persons in Kenya were HIV positive by the end of 2022, with 22,000 new infections and 18,000 fatalities attributable to the virus [(4)].

The HIV virus earmarks and infects mostly CD4+ T helper lymphocytes, the cluster of differentiation 4-positive (CD4+) cells [5]. As soon as the virus infects CD4+ cells, the host immune system goes into overdrive, releasing inflammatory cytokines and chemokines, activating polyclonal B-cells, and gradually reducing the number of CD4+ T-cells. Erythrocytes and other CD4 negative blood cells may bind to the virus when complement receptor type 1 (CR1), also called the C3b/C4b receptor or CD35, is present [(6,7)]. The infection always causes a gradual decrease of CD4+ T cells because the host cell dies at the end of the infection process [(8)]. When CD4+ T cells interact with B cells, CD8+ T cells, antigen presentation cells (APCs), and natural killer cells, the body is able to eliminate invading pathogens [(9)]. When CD4+ T cells, which are responsible for regulating the adaptive immune system, are reduced in number, the immune system becomes less efficient and eventually leads to acquired immune deficiency syndrome (AIDS)[(5)]

HIV infection has been linked to several immunological and haematological disorders. CD4+ T cells decline, cytokine

imbalance, and immune system dysfunction are the main immunological concerns in people with HIV. The progression of HIV causes a cascade of symptoms, the most common of which are haematological abnormalities [10-12]. Regardless of the stage HIV infection is in its progression, these anomalies are a leading cause of death and disability among those living with the virus [13]/. Haematological disorders include faulty hematopoiesis, coagulation system abnormalities and cytopenias affecting different types of blood cells [14]. The varied causes for these abnormalities include the HIV virus itself, immune-mediated cell death, neoplasms, opportunistic infections, and the harmful effects of antiretroviral drugs **[14,15].** Most hematological abnormalities fall under the category of peripheral cytopenias, which include anemia, leucopenia, and thrombocytopenia [16]. Because of these anomalies, the quality of life of those living with HIV is greatly diminished, and the rates of illness and death are also higher **[17].**

An important part of HIV management is antiretroviral medication (ART), which helps keep the virus under control, keeps the immune system functioning, stops transmission, and treats other health related issues **[18]**. Highly active antiretroviral therapy (HAART) is an essential part of antiretroviral treatment (ART). It consists of three or more antiretroviral medications. Some examples of these are protease inhibitors, nucleoside reverse transcriptase inhibitors, and nucleotide reverse transcriptase inhibitors. When used effectively, HAART improves health outcomes by reducing viral replication, increasing the immunological response, preventing drug resistance, and delaying the development of HIV to AIDS **[19]**. Negative responses to HAART include gastrointestinal, neurological, hematological, psychological, and metabolic problems; hypersensitivity; oral ulcers; fever; and menstrual abnormalities; and these side effects have been linked to the treatment, despite its efficacy **[20-22].**

All people infected with HIV should start combination antiretroviral treatment (cART) promptly, regardless of their clinical, immunological, or virological state; by 2018, Kenya had embraced these 2015 World Health Organization recommendations known as Universal "test-and-treat" (UTT) strategy **[23].** In addition, as of 2018, Kenya's ARV guidelines featured both DTG and AZT containing regimens as the preferred first line and second line options for adult HIV infected patients **[24]**. As of 2022, approximately 1,297,822 Kenyans living with HIV were receiving ART, a 94% ART coverage and about 1200 000 (89%) of people living with HIV on HAART had suppressed vial loads **[25]**. "Recognizing that antiretroviral therapy (ART) can lead to various side effects and may influence haematological parameters both positively and negatively, it is crucial to consistently monitor the blood cell levels of HIV-infected patients undergoing treatment. This monitoring allows for the early identification of haematological and immunological anomalies and facilitates timely clinical interventions. However, there is limited data on how different ART regimens affect the haematological and immunological parameters in HIV-infected adults in our setting. Therefore, this study aims to evaluate the haematological and immunological abnormalities in HIV-infected patients, comparing the effects of various ART regimens at Thika Level Five Hospital Comprehensive Care Centre in Kiambu County, Kenya.

Materials and Methods

Study Location

The HIV Comprehensive Care Centre at Thika Level Five Hospital in Kiambu County, Kenya, was the site of this study. Located in the Biashara sub-location along General Kago Road in the Thika West District of the Thika Municipality Division, Thika Level Five Hospital is a county referral institution. Located around 50 miles northeast of Nairobi in Central Province, the hospital has a capacity of 467 beds. With 2,417,735 inhabitants and an area of 2,539 km², Kiambu County has a population density of 952.4/km², making it the second most populated county in Kenya, after Nairobi.

Thika Level Five Hospital serves as an inter-county referral facility for a wide catchment area, including Machakos, Kitui, Murang'a, and Nairobi. Offering comprehensive services for HIV/TB prevention, care, and treatment, it is recognized as a Centre of Excellence. This encompasses a wide range of services related to maternal and child health, including HIV testing, care, and treatment, and the prevention of mother-to-child transmission of the virus.

Besides, existing data shows that Kiambu County has an estimated 59,016 people living with HIV, with an overall prevalence of 4.0%, with 2.1% prevalence in men and 5.9% in women. By 2018, approximately 34,417 adults and 1,972 children were on ART, with ART coverage of 61% and 82% for adults and children, respectively 12.

Study Design, Period, and Population

Thika Level Five Hospital Comprehensive Care Centre was the site of this cross-sectional study, which ran from July 2022 through December 2023. HIV-positive patients who were newly diagnosed as well as those patients who were already receiving treatment or care at Thika Level Five Hospital CCC and satisfied the eligibility requirements were included in the study. The population was divided into 106 ART-exposed and 51 ART-naïve groups, with each clinic visitor during the study period being evaluated for eligibility. A control group of 80 HIV negative individuals was also enrolled into the study.

Inclusion Criteria

HIV-infected individuals aged 18 and older who were willing to give their consent were included in the study.

Exclusion Criteria

Exclusion criteria included (i) HIV patients with severe concomitant diseases (e.g., TB, cancer), (ii) those with known haematological diseases/disorders, (iii) individuals on medications other than cotrimoxazole prophylaxis, "patients who had undergone a blood transfusion in the past three

months, (v) those using vitamins and iron supplements during enrollment, (vi) pregnant women, (vii) nursing mothers, and (viii) individuals who do not agree to participate in the study."

Sample Size and Sampling Method

Using a population proportionate calculation, the sample size was determined with a goal of a 95% confidence interval and a 5% margin of error. This was based on a previous research that found a 16% prevalence of cytopenia and a total population of 56,622.

Formula
n = N*X / (X + N – 1)
where,
X = Zα/22 ¬*p*(1-p) / MOE2,
n = N*X / (X + N – 1),
A minimum sample size of 206 participants was reached by substituting the values.
There were a total of 237 participants after removing the 15% non-response rate.
Sample Size = 237 Subjects

Data Collection

In addition to reviewing medical records, we used a pretested structured questionnaire to collect socio-demographic information and clinical traits from research participants. Data collection was conducted by a clinician at the ART clinic, two experienced laboratory technologists, and the principal investigator, who supervised the entire process.
Afterwards, each participant had 4 milliliters of venous blood samples drawn in EDTA anticoagulant. The samplke was used for testing for full blood counts and immunological CD4 counts.

Laboratory Procedures

Haematological and CD4 Count Analysis: Complete blood count (CBC) testing was performed using the DYMIND DF-52 automated haematology analyser (DYMIND BIOTECH, China). The analyser provided data on various blood parameters, including WBCs, RBCs, lymphocytes, neutrophils, haemoglobin levels, and platelet counts. CD4+ T cell counts were measured using the Becton Dickinson FACS Calibur system (Becton Dickinson, Singapore).

Operational Definitions

The World Health Organization's criteria were used for defining and grading anaemia [(26)]. When the hemoglobin level was less than 13 g/dL in men, it was classified as mild anemia, moderate anemia, or severe anemia. A woman was considered to have anaemia if her hemoglobin level was less than 12.0 g/dL for mild, 8.0-10.9 g/dL for moderate, and less than 8.0 g/dL for severe. Leucopoenia was characterized as a white blood cell count below 4.0 × 10^3/μL, while a normal white blood cell count was 4-11 × 10^3/μL and a leucocytosis count over 11 × 10^3/μL was declared. As ANC <1.5 × 10^3/μL, neutropenia was deemed to have occurred. In this study, lymphopenia was defined as an ALC level below 1 × 10^3/μL. The condition known as thrombocytopenia was identified when the platelet count was less than 150 × 10^3/μL. It was further categorized as mild (100-149 × 10^3/μL), moderate (50-99 × 10^3/μL), and severe (<50 × 10^3/μL). Different types of anemia were categorized as normocytic, microcytic, macrocytic, normochromic, and hypochromic, based on the mean corpuscular volume (MCV) (80-100 fL), 80 < fL, >100 fL, and >27 pg, respectively). The CDC staging method divided HIV-infected individuals into three categories according to CD4 counts: stage 1 (>500 cells/mm^3), stage 2 (200-499 cells/mm^3), and stage 3 (<200 cells/mm^3).

Data Quality Control

Specimen collection and testing were conducted in strict accordance with laboratory standard operating procedures (SOPs) to ensure data quality. The questionnaire was reviewed by the principal investigator to ensure they were valid and was also pre-tested before the actual data collection. Performance of the automated haematology analyser used for haematological analysis and FACS Calibur system used for CD4 count was checked daily by running the three levels of quality control samples that is low, normal and high before and during the analysis of the patient samples. Routine quality control checks were performed according to manufacturer instructions. Data collectors were trained before the study, with regular supervision and follow-up by the principal investigators. To make sure the data was consistent and comprehensive, it was examined every day.

Statistical Analysis

For analysis, the data was exported to GraphPad Prism version 10.2, after being coded, cleaned, and updated. Immunohaematological parameters across the study groups were compared using unpaired T – test with Welch's correction. A P-value less than 0.05 was regarded statistically significant. Fisher's exact test was used to determine relationship between immunohaematological abnormalities and ART regimen.

Ethical Considerations

This study has received the green light from Mount Kenya University's Institutional Ethics Review Board (MKU/ERC/1013). The administration of Thika Level Five Hospital granted approval, and further authority was sought from the Health Research and Development Unit of Kiambu County. The goals of the study, the importance of maintaining their privacy, and the fact that their data will be used only for research were all communicated to the study participants in detail. All participants gave their written informed permission before blood samples were taken.

Results

Socio-demographic and clinical Characteristics of the Study Participants

Table 1 show the demographic and health information of the study subjects. The research comprised 237 participants, 122 of whom were male and 115 of whom were female. A total of 106 people with HIV who were receiving antiretroviral therapy (ART), 51 individuals with HIV who were ART-naive, and 80 adult controls who tested negative for HIV made up the participants.

The participants included newly diagnosed HIV infected individuals prior to ART initiation or those who had been on ART treatment and care for at least six months at the Thika Level Five Hospital Comprehensive Care Center (CCC) between July 2022 and December 2024. Out of the total 237 study participants 122 (51.48%) were males compared to 115 females (48.52%), with a male-to-female ratio of 1.06:1. The ages of the study participants ranged from 18 to 66 years, with majority of the participants (88.18%) being 50 years or younger. The predominant age group was 18-25 years (31.65%), and the age bracket of 66 years and above was the least (0.42%). Most of the study participants were single (57%), with 43.88% married, 6.33% were divorced, and only 4.22% were widowed. Majority of the participants (65.1%) had a normal body mass index (BMI), while 3.36% were underweight, 28.18% overweight, and 3.36% obese.

The study participants were taking any of the five different ART regimens with majority of them (79.25%) being placed on TDF/3TC/DTG (TLD) regimen. Those who were on TDF-based ART regimens were more (90.57%) than those on AZT – based regimens (8.49%) and ABC- based regimens (0.94%). Most of the study participants (76.42%) were on ART treatment for more than 6 yrs.

Results on **Table 2** shows the mean values of the haematological and immunological parameters of the ART – treated, ART – naïve. The mean ± SD of the PLT, ALC, HB, MCV, MCH, and PCV had a statistically significant difference between the HIV infected individuals who had been treated with antiretroviral therapy (ART) and those who had not.

The mean ± SD of PLT, ALC, HB, MCV, MCH, PCV, of ART – treated was significantly higher compared with mean ± SD of ART – naïve; 410.80±217.10 vs 309.30±147.90 (p =0.0030), 2.03±0.66 vs 1.50±0.66 (p <0.0001), 13.96± 2.01 vs 12.63±2.99 (p =0.0054), 90.35±10.48 vs 82.92±9.42 (p <0.0001), 27.76±3.18 vs 25.83±3.50 (p =0.0013), 45.53±6.29 vs 40.57±9.13 (p =0.0008) respectively. Despite that, the mean ± SD of the WBC, RBC, ANC, AMC, RDW and CD4 count of ART- treated and ART- naïve were not significantly different; 5.40±1.46 vs 5.24±2.71(p =0.6289), 5.05±0.61 vs 4.80±1.13 (p =0.1615), 2.89±1.48 vs 3.55±2.55 (p =0.0901), 0.41±0.13 vs 0.43±0.32 (p =0.6676), 14.44±2.00 vs 15.07±2.20 (p =0.0855), 378.50±317.7 vs 348.50±256.90 (p =0.5045) as shown in **Table 2**

"Participants in the study were treated with five different types of HAART regimens, including zidovudine/lamivudine/atazanavir/ritonavir(AZT/3TC/ATV/r), tenofovir/lamivudine/dolutegravir (TDF/3TC/DTG),tenofovir/lamivudine/atazanavir/ritonavir(TDF/3TC/ATV/r), abacavir/lamivudine/atazanavir/ritonavir(ABC/3TC/ATV/r),and zidovudine/lamivudine/dolutegravir (AZT/3TC/DTG)."The influence of the ART regimens on the hematological and immunological abnormalities was assessed (**Table 3).** The ART regimen significantly influenced ALC, HCT, MCV, MCH, RDW, and HIV disease stage abnormalities. Lymphopenia patients on TDF/3TC/DTG were more likely to experience it than those on AZT/3TC/ATV/r or ABC/3TC/ATV/r regimens (p=0.0252).

Low HCT was significantly higher in patients on AZT/3TC/ATV/r (100.00%, p<0.0001), while high HCT was more frequent in patients on TDF/3TC/DTG (53.85%, p<0.0001). Microcytosis was significantly higher in those on TDF/3TC/DTG (100.00%, p<0.0001), and macrocytosis was more prevalent in patients on AZT/3TC/ATV/r (35.29%, p<0.0001). Hypochromia was significantly higher in those on TDF/3TC/DTG (94.12%, p=0.0268). Elevated RDW was more frequent in patients on TDF/3TC/DTG (60.00%, p=0.0044). The ART regimen significantly influenced the HIV disease stage, with higher Stage I prevalence in patients on AZT/3TC/DTG (81.82%, p<0.0001) and higher Stage II and III prevalence in those on TDF/3TC/DTG (77.36% and 87.50%, respectively, p<0.0001). The ART regimen did not significantly influence WBC, RBC, PLT, ANC, AMC, and HB abnormalities **(Table 3).**

Table 1: Socio-demographic data of the study population

Patient Factor		Number (n)	Percentage (%)
Gender (n = 237)	Male	122	51.48
	Female	115	48.52
Age group (Years) (n = 237)	18-25	75	31.65
	26-35	64	27.00
	36-45	53	22.36
	46-55	27	11.39
	56-65	17	7.17
	≥66	1	0.42
Marital status (n = 237)	Single	108	45.57
	Married	104	43.88
	Divorced	15	6.33
	Widowed	10	4.22
BMI (Kg/M^2)	<20	26	16.56
(HIV +ve only; n = 157)	21-25	84	53.50
	26-30	42	26.75
	>30	5	3.18
HAART regimen	AZT/3TC/ATV/r	7	6.60%
	TDF/3TC/DTG	84	79.25
	TDF/3TC/ATV/r	12	11.32%
	ABC/3TC/ATV/r	1	0.95%
	AZT/3TC/DTG	2	1.88%
HAART duration	6 months - 5 Yrs	25	
	6-10 Yrs	41	
	11-15 Yrs	33	
	16- 20 Yrs	7	

Table 2: Comparison of Haematological and Immunological Parameters between ART- Treated and ART-Naive

Parameter	ART-Treated	ART-Naïve	*t* (df)	P value
WBC ($\times 10^3/\mu L$)	5.40±1.46	5.24±2.71	0.4843 (155)	0.6289
RBC ($\times 10^3/\mu L$)	5.05±0.61	4.80±1.13	1.407 (155)	0.1615
PLT count ($\times 10^3/\mu L$)	410.80±217.10	309.30±147.90	3.016 (155)	0.0030
ANC ($\times 10^3/\mu L$)	2.89±1.48	3.55±2.55	1.720 (66.26)	0.0901
ALC ($\times 10^3/\mu L$)	2.03±0.66	1.50±0.66	4.643 (98.57)	<0.0001
HB (g/dL)	13.96± 2.01	12.63±2.99	2.868 (72.41)	0.0054
MCV (fL)	90.35±10.48	82.92±9.42	4.461 (109.0)	<0.0001
MCH (pg)	27.76±3.18	25.83±3.50	3.3290 (90.85)	0.0013
PCV (%)	45.53±6.29	40.57±9.13	3.50 (73.62)	0.0008
AMC ($\times 10^3/\mu L$)	0.41±0.13	0.43±0.32	0.4317 (58.43)	0.6676
RDW	14.44±2.00	15.07±2.20	1.7390 (90.83)	0.0855
CD_4 count (cells/μL)	378.50±317.7	348.50±256.90	0.6697 (108.4)	0.5045

Values are presented as $\overline{x} \pm SD$. Unpaired student t-tests with Welch's correction at $\alpha_{0.05}$

Table 3: Hematological and immunological abnormalities of ART - treated patients in relation to the type of ART regimen

Abnormality if present		ART Regimen					P value
		AZT/3TC/ATV/r	TDF/3TC/DTG	TDF/3TC/ATV/r	ABC/3TC/ATV/r	AZT/3TC/DTG	
WBC	Leucopenia (n= 19)	1 (5.26 %)	17 (89.47 %)	0 (0.00%)	1 (5.26%)	0 (0.00%)	0.2475
	Normal (n= 85)	5 (5.88 %)	66 (77.65 %)	12 (14.12 %)	0 (0.00 %)	2 (2.35 %)	
	Leukocytosis (n= 1)	0 (0.00 %)	1 (100.00 %)	0 (0.00 %)	0 (0.00 %)	0 (0.00 %)	
RBC	Erythropenia (n= 1)	1 (33.33 %)	2 (66.67 %)	0 (0.00 %)	0 (0.00 %)	0 (0.00 %)	0.5214
	Normal RBC (n= 5)	0 (0.00 %)	5 (100 %)	0 (0.00 %)	0 (0.00 %)	0 (0.00 %)	
	Erythrocytosis (n=98)	6 (6.12 %)	77 (78.57 %)	12 (12.24 %)	1 (1.02 %)	2 (2.04 %)	
PLT	Mild thrombocytopenia (n= 0)	0 (0.00 %)	0 (0.00 %)	0 (0.00 %)	0 (0.00 %)	0 (0.00 %)	0.0800
	Moderate thrombocytopenia (n= 1)	0 (0.00 %)	1 (100.00 %)	0 (0.00 %)	0 (0.00 %)	0 (0.00 %)	
	Normal PLT (n = 76)	6 (7.89 %)	56 (73.68 %)	12 (15.79 %)	1 (1.32 %	1 (1.32 %)	
	Thrombocytosis (n= 28)	0 (0.00 %)	27 (96.43 %)	0 (0.00 %)	0 (0.00 %)	1 (3.57)	
ANC	Neutropenia (n=12)	0 (0.00 %)	12 (100 %)	0 (0.00 %)	0 (0.00 %)	0 (0.00 %)	0.7537
	Moderate neutropenia (n= 4)	0 (0.00 %)	4 (100 %)	0 (0.00 %)	0 (0.00 %)	0 (0.00 %)	
	Normal neutrophil (n =84)	6 (7.14 %)	64 (76.19 %)	11 (13.10 %)	1 (1.19 %)	2 (2.38 %)	
	Neutrophilia (n =5)	1 (20.00%)	4 (80.00 %)	0 (0.00 %)	0 (0.00 %)	0 (0.00 %)	
ALC	Lymphopenia (n= 4)	1 (25.00%)	2 (50.00 %)	0 (0.00 %)	1 (25.00%)	0 (0.00 %)	0.0252
	Normal (n= 101)	6 (5.94 %)	82 (81.19 %)	11 (10.89 %)	0 (0.00 %)	2 (1.98 %)	
AMC	Normal (n =103)	6 (5.83 %)	83 (80.58 %)	11 (10.68 %)	1 (0.97 %)	2 (1.94 %)	>0.9999
	Monocytosis (n =1)	0 (0.00 %)	1 (100.00 %)	0 (0.00 %)	0 (0.00 %)	0 (0.00 %)	
Hb	Severe anemia (n= 2)	0 (0.00 %)	2 (100.00 %)	0 (0.00 %)	0 (0.00 %)	0 (0.00 %)	0.8660
	Mild anemia (n= 7)	0 (0.00 %)	7 (100.00 %)	0 (0.00 %)	0 (0.00 %)	0 (0.00 %)	
	Moderate anemia (n = 6)	1 (16.67 %)	5 (83.33 %)	0 (0.00 %)	0 (0.00 %)	0 (0.00 %)	
	Normal Hb (n= 89)	6 (6.74 %)	70 (78.65 %)	10 (11.24 %)	1 (1.12 %)	2 (2.25 %)	
HCT	Low HCT (n =12)	12 (100.00 %)	0 (0.00 %)	0 (0.00 %)	0 (0.00 %)	0 (0.00 %)	<0.0001
	Normal HCT (n =79)	3 (3.80 %)	65 (82.28 %)	10 (12.66 %)	0 (0.00 %)	1 (1.27 %)	

Abnormality if present		ART Regimen					P value
	High HCT (n =13)	4 (30.77 %)	7 (53.85 %)	0 (0.00 %)	1 (7.69 %)	1 (7.69 %)	
MCV	Microcytosis (n =17)	0 (0.00 %)	0 (100.00 %)	0 (0.00 %)	0 (0.00 %)	0 (0.00 %)	<0.0001
	Normocytosis (n= 72)	1 (1.39 %)	61 (84.72 %)	10 (13.89 %)	0 (0.00 %)	0 (0.00 %)	
	Macrocytosis (n= 17)	9 (52.94 %)	5 (29.41 %)	(0.00 %)	1 (5.88 %)	2 (11.76 %)	
MCH	Normochromia (n =71)	4 (5.63 %)	52 (73.24 %)	12 (16.90 %)	1 (1.41 %)	2 (2.82 %)	0.0268
	Hypochromia (n =34)	2 (5.88 %)	32 (94.12 %)	0 (0.00 %)	0 (0.00 %)	0 (0.00 %)	
RDW	Normal RDW (n = 95)	3 (3.16 %)	78 (72.11 %)	12 (12.63 %)	0 (0.00 %)	2 (2.11 %)	0.0044
	High RDW (n= 10)	3 (30.00%)	6 (60.00 %)	0 (0.00 %)	1 (10.00 %)	0 (0.00 %)	
CD_4 count	Stage I (CD4>500 cells/µL) (n =44)	2 (4.55 %)	0 (0.00 %)	6 (13.64 %)	0 (0.00 %)	36 (81.82 %)	<0.0001
	Stage II (CD4 200 to 499 cells/µL (n =53)	4 (7.55 %)	41 (77.36 %)	6 (11.32 %)	0 (0.00 %)	2 (3.77 %)	
	Stage III (CD4< 200 cells/µL (n= 8)	0 (0.00 %)	7 (87.50 %)	0 (0.00 %)	1 (12.50 %)	0 (0.00 %)	

Fisher's exact test; $\alpha_{0.05}$

Discussion

At the Comprehensive Care Centre at Thika Level Five Hospital in Kiambu County, Kenya, persons living with HIV were studied to determine the effect of different antiretroviral therapy regimens on hematological abnormalities and immunological CD4 cell counts. The most frequent hematological abnormalities in HIV-positive people are cytopenias, which include anemia, leucopenia, and thrombocytopenia [27, 28]. To achieve prolonged suppression of viral load, improve quality of life, restore and preserve immunological function, and reduce HIV-related morbidity and death are the core therapeutic goals of antiretroviral therapy (ART) [28]. However, the use of antiretroviral therapy has been associated with adverse drug reactions, including hematologic toxicity, which can lead to hematological abnormalities, particularly cytopenias, as well as liver toxicity and other side effects[29,30,31,32]. As haematological abnormalities frequently pose a health challenge to the HIV-infected individuals, assessment of these abnormalities during antiretroviral treatment is crucial so as to evaluate the patient's response to antiretroviral therapy.

Initiation of antiretroviral medication may have restored hematopoiesis in the bone marrow, which might explain the noticeably higher levels of PLT, ALC, HB, MCV, MCH, and PCV in the ART-treated group as compared to the ART-naïve group ($p < 0.05$). The favorable effects of HAART on RBC differentiation and survival, viral load control, and a reduction in the incidence of opportunistic infections are thought to be responsible for the decrease in anemia that occurs after commencing the therapy [33, 34].

The normal range of CD4 count is 500 -1500 cells/mm3. CD4 count is used to monitor the effectiveness of the antiretroviral treatment (ART), monitor disease progression, used in HIV disease staging and guiding medical therapy. The CDC states that a CD4 cell count below 200 cells/mm³ is one aspect that must be considered when diagnosing AIDS. Opportunistic infections and higher death rates are linked to a decline in CD4 T cells [35]. The mean ± SD CD4 count of the ART-treated patients was higher than that of the ART – naive though no statistically significant difference between the two study groups ($p = 0.5045$). However, the findings of the present study show that only 7.54% of the ART – treated HIV-infected individuals had CD4 count < 200 cells/µL. This is an indication of the effectiveness of the ART regimens in immune recovery and improvement in the number of CD4+ T cells in HIV- infected patients on Antiretroviral therapy.

Specific ART regimens demonstrated significant associations with haematological abnormalities, such as lymphopenia, HCT, MCV, MCH, RDW, and CD4 count abnormalities. For instance, the TDF/3TC/DTG regimen associated with higher lymphopenia rates, emphasizing the importance of regimen selection in managing haematological complications [36, 37]. High rates of macrocytosis were seen in patients on AZT/3TC/ATV/r (35.29%, $p<0.0001$) an AZT- containing regimen, which is in agreement with other previous studies that have reported development of macrocytosis in patients on AZT-containing regimens ([38, 39]. Moreover, the association

between AZT-containing regimens and macrocytosis underscores the need for monitoring and potential interventions to mitigate adverse effects [38, 40]. These findings underscore the importance of considering individual patient characteristics and drug interactions when selecting ART regimens. Furthermore, exploring the potential role of newer ART agents in reducing haematological toxicity warrants further investigation to enhance treatment tolerability and efficacy.

Conclusion and recommendations

Some of the haematological parameters such as PLT, ALC, HB, MCV, MCH, PCV for the ART-treated were significantly higher than those of ART-naïve individuals. The ART regimen significantly influenced the PLT, ALC, HCT, MCV, MCH, RDW, and HIV disease stage abnormalities of the ART- treated HIV – infected patients. Lymphopenia was significantly higher in patients on TDF/3TC/DTG regimens. There were significantly higher rates of macrocytosis in patients on AZT/3TC/ATV/r.

Patients with HIV who are receiving antiretroviral treatment may benefit from this study's basic data on the effects of various ART regimens on immunological and haematological abnormalities. The findings highlight the necessity for individualized monitoring and treatment plans that take into account the type of ART regimens. Future research should concentrate on large scale longitudinal studies in order to better comprehend the long-term effects of ART on haematological parameters and identify novel treatment strategies to reduce unfavorable outcomes. The study emphasizes the value of routine monitoring of patients on ART for haematological abnormalities, stressing the necessity for healthcare providers to be watchful of adverse effects of antiretroviral therapy.

Data availability:

The datasets used in this work are not publicly accessible, however they may be requested from the corresponding author in accordance with the ethical reviewer's rules.

Conflict of interest:

Regarding the study, writing, and publishing of this work, the author(s) hereby affirm that they have no possible conflicts of interest.

Author contributions

EWM, conceptualized the idea, structured the study, oversaw the research and wrote the first draft. SWK and SE helped develop it, while GM analyzed the data. After reading and revising the final document, all authors gave their approval for submission.

Acknowledgements

To the clinicians and lab workers at Thika Level Five Hospital CCC who helped with data collection and processing, as well as to the laboratory personnel at Thika Blood Satellite Center and Thika Level Five Hospital Laboratory, the authors are eternally grateful. The researchers would also want to thank the people who took part in the study and gave their consent to provide data and samples for the research. Our utmost gratitude goes to Gervason Moriasi for performing data analysis, Mr. Jeremiah Thiongo the clinician at The Thika Level Five Hospital Comprehensive Care Centre and Ms. Lucy Wamuyu, laboratory manager at Thika Level Five Hospital Laboratory and Mr. Geoffrey Githinji Muthee, the Program Officer, HIV Service – NASCOP for their unwavering support.

REFERENCES

1. Blood, G. A. C. (2016). Human immunodeficiency virus (HIV). *Transfusion medicine and hemotherapy*, *43*(3), 203.
2. World Health Organization. *HIV and AIDS: key facts*. World Health Organization. Published 2023. Accessed August 7, 2023. https://www.who.int/news-room/fact-sheets/detail/hiv-aids. [Google Scholar].
3. Joint United Nations Programme on HIV/AIDS. The path that ends AIDS: UNAIDS Global AIDS Update 2023. Joint United Nations Programme on HIV/AIDS. Published 2023. Accessed August 5, 2023. https://www.unaids.org/en/resources/documents/2023/global-aids-update-2023. [Google Scholar]
4. UNAIDS, Global HIV. & AIDS statistics — Fact sheet| UNAIDS [Internet]. 2022 [cited 2022 Oct 21]. Available from: https://www.unaids.org/en/resources/fact-sheet.
5. Vidya Vijayan, K.K.; Karthigeyan, K.P.; Tripathi, S.P.; Hanna, L.E. Pathophysiology of CD4+ T-Cell Depletion in HIV-1 and HIV-2 Infections. *Front. Immunol.* **2017**, *8*, 580. [**Google Scholar**] [**CrossRef**]
6. Horakova E, Gasser O, Sadallah S, et al. Complement mediates the binding of HIV to erythrocytes. J Immunol 2004; 173(6): 4236–4241.
7. Gudina EK, Teklu AM, Berhan A, et al. Magnitude of antiretroviral drug toxicity in adult HIV patients in Ethiopia: a cohort study at seven teaching hospitals. Ethiop J Health Sci 2017;27(Suppl. 1): 39–52.
8. Fenwick, C.; Joo, V.; Jacquier, P.; Noto, A.; Banga, R.; Perreau, M.; Pantaleo, G. T-Cell Exhaustion in HIV Infection. *Immunol. Rev.* **2019**, *292*, 149–163. [**Google Scholar**] [**CrossRef**] [**PubMed**]
9. Mohamad WMW, Ab Rahman WSW, Al-Salih SAA, Hussin CMC. Immunological and Haematological Changes in HIV Infection. In: *Trends in Basic and Therapeutic Options in HIV Infection - Towards a Functional Cure*. InTech; 2015. doi:10.5772/61259
10. Dikshit B, Wanchu A, Sachdeva RK, Sharma A, Das R. Profile of hematological abnormalities of Indian HIV infected individuals. BMC Hematol. 2009;9(1):1–6. doi:10.1186/1471-2326-9-5
11. Kirchhoff F, Silvestri G. Is Nef the elusive cause of HIV-associated hematopoietic dysfunction? J Clin Invest. 2008;118(5):1622–1625. doi:10.1172/JCI35487
12. Dhurve SA, Dhurve AS. Bone marrow abnormalities in HIV disease. Mediterr J Hematol Infect Dis. 2013;5(1):e2013033. doi:10.4084/ mjhid.2013.033

13. Muluneh A, Fessahaye A. Hematologic abnormalities among children on HAART in Jimma University specialized hospital, Southwestern Ethiopia. Ethiop J Health Sci. 2009;19(2):83–89
14. Comprehensive, up-to-date information on HIV/AIDS treatment and prevention from the University of California San Francisco. HIV Transmission and Prevention in Adolescents. http://hivinsite.ucsf.edu/InSite?page=kb-04-01-09. Accessed March 6, 2019.
15. Wankah PN, Tagny CT, Mbanya DNS. Profile of blood cell abnormalities among antiretroviral therapy naïve HIV patients attending the Yaounde University Teaching Hospital, Cameroon. *BMC Hematol*. 2014;14(1). doi:10.1186/2052-1839-14-15
16. Redig AJ, Berliner N. Pathogenesis and clinical implications of HIV-related anemia in 2013. Hematology Am Soc Hematol Educ Program. 2013;2013:377-81. doi: 10.1182/asheducation-2013.1.377. PMID: 24319207.
17. Kathuria S, Bagga PK, Malhotra S. Hematological manifestations in HIV infected patients and correlation with Cd4 counts and anti retroviral therapy. J Contemp Med Res 2016;3:3495–8.
18. Rossi, E., Meuser, M. E., Cunanan, C. J., & Cocklin, S. (2021). Structure, Function, and Interactions of the HIV-1 Capsid Protein. Life, 11(2), 100. https://doi.org/10.3390/life11020100
19. Hileman, C. O., & Funderburg, N. T. (2017). Inflammation, Immune Activation, and Antiretroviral Therapy in HIV. Current HIV/AIDS reports, 14(3), 93–100. https://doi.org/10.1007/s11904-017-0356-x
20. Nagpal M, Tayal V, Kumar S, Gupta U: Adverse drug reactions to antiretroviral therapy in AIDS patients at a tertiary care hospital in India: A prospective observational study. Indian J Med Sci. 2010, 64:245–52. 10.4103/0019-5359.99597
21. Tadesse WT, Mekonnen AB, Tesfaye WH, Tadesse YT: Self-reported adverse drug reactions and their influence on highly active antiretroviral therapy in HIV infected patients: a cross sectional study. BMC Pharmacol Toxicol. 2014, 15:32. Accessed: December 28, 2015 http://bmcpharmacoltoxicol.biomedcentral.com/articles/10.1186/2050-6511-15-32. 10.1186/2050-6511-15-32
22. Balakrishnan, A., Valsalan, R., Sheshadri, S., Pandit, V., Medep, V., & Agrawal, R. "Zidovudine-induced reversible pure red cell aplasia." *Indian Journal of Pharmacology*, vol. 42, no. 3, 2010, p. 189. Accessed 17 Nov. 2020.
23. World Health Organization. WHO Guideline on when to start antiretroviral therapy and on pre-exposure prophylaxis for HIV September 2015 [27th Dec, 2019]. https://apps.who.int/iris/bitstream/handle/10665/186275/9789241509565_eng.pdf;jsessionid=D95F5690AC992DCB82B673779D9EF5D9?sequence=1
24. Ministry of Health, National AIDS & STI Control Program. Guidelines on Use of Antiretroviral Drugs for Treating and Preventing HIV Infection in Kenya 2018 Edition. Nairobi, Kenya: NASCOP, August 2018. Print.
25. Joint United Nations Program on HIV/AIDS (UNAIDS). *Country Fact Sheets: Kenya*; 2022. Available at: https://www.unaids.org/en/regionscountries/countries/kenya Accessed July 17, 2022.
26. WHO. Haemoglobin Concentrations For the Diagnosis of Anaemia and Assessment of severity. Vitamin and Mineral Nutrition Information System. Geneva: World Health Organization; 2011. (WHO/NMH/NHD/MNM/11.1); http://www.who.int/vmnis/indicators/haemoglobin. pdf. Accessed 20 May 2021.
27. Shen Y, Wang J, Wang Z, et al. A cross-sectional study of leukopenia and thrombocytopenia among Chinese adults with newly diagnosed HIV/AIDS. Biosci Trends. 2015;9(2):91–96. doi:10.5582/ bst.2015.01024
28. Vishnu, P., & Aboulafia, D. M. (2015). Haematological manifestations of human immune deficiency virus infection. British Journal of Haematology, 171(5), 695–709. https://doi.org/10.1111/bjh.13783
29. Balakrishnan A et al. zidovudine-induced reversible pure red cell aplasia. Indian J Pharmacol.Jun 2010; 42(3) 189 - 191.
30. Enawgaw B, Alem M, Addis Z, Melku M (2014) Determination of hematological and immunological parameters among HIV positive participants taking highly active antiretroviral treatment and treatment naïve in the antiretroviral therapy clinic of Gondar University Hospital, Gondar, Northwest Ethiopia: A comparative cross-sectional study. BMC Hematology 14:
31. Nuñez MJ, Martin-Carbonero L, Moreno V, Valencia E, Garcia-Samaniego J, et al. (2006) Impact of antiretroviral treatment-related toxicities on hospital admissions in HIV-infected participants. AIDS Res Hum Retroviruses 22: 825-829.
32. Kovari H, Sabin CA, Ledergerber B, Ryom L, Reiss P, et al. (2016) Antiretroviral drugs and risk of chronic alanine aminotransferase elevation in human immunodeficiency virus (HIV)-monoinfected persons: The data collection on adverse events of anti-HIV drugs study. Open Forum Infectious Diseases 3: ofw009.
33. Fokouo, J. V. F., Vokwely, J. E. E., Noubiap, J. J. N., Nouthe, B. E., Zafack, J., Minka Ngom, E. S., Dalil, A. B., Ngo Nyeki, A. R., Bengono, G., & Njock, R. (2015). Effect of HIV infection and highly active antiretroviral therapy on hearing function: A prospective case-control study from Cameroon. JAMA Otolaryngology - Head and Neck Surgery, 141(5), 436–441. https://doi.org/10.1001/jamaoto.2015.125
34. Denue, B. A., Kida, I. M., Hammagabdo, A., Dayar, A., & Sahabi, M. A. (2013b). Prevalence of Anemia and Immunological Markers in HIV-Infected Patients on Highly Active Antiretroviral Therapy in Northeastern Nigeria. Infectious Diseases: Research and Treatment, 6, IDRT.S10477. https://doi.org/10.4137/idrt.s10477

35. Battistini Garcia, S. A., & Guzman, N. (2023). Acquired Immune Deficiency Syndrome CD4+ Count. In StatPearls. StatPearls Publishing.
36. Fekene TE, Juhar LH, Mengesha CH, Worku DK. Prevalence of cytopenias in both HAART and HAART naïve HIV infected adult patients in Ethiopia: A cross sectional study. BMC Hematol. 2018;18(1). doi:10.1186/s12878-018-0102-7
37. Enawgaw B, Alem M, Addis Z, Melku M. Determination of hematological and immunological parameters among HIV positive patients taking highly active antiretroviral treatment and treatment naïve in the antiretroviral therapy clinic of Gondar University Hospital, Gondar, Northwest Ethiopia: A comparative cross-sectional study. BMC Hematol. 2014;14(1):8. doi:10.1186/2052-1839-14-8
38. Parkes-Ratanshi, R., Katende, D., Levin, J., Wakeham, K., Heiner, G., Kamali, A., & Lalloo, D. G. (2015). Development of Severe Anemia and Changes in Hemoglobin in a Cohort of HIV-Infected Ugandan Adults Receiving Zidovudine-, Stavudine-, and Tenofovir-Containing Antiretroviral Regimens. Journal of the International Association of Providers of AIDS Care, 14(5), 455–462. https://doi.org/10.1177/2325957414557264
39. Tadele A. Prevalence of Zidovudine induced megaloblastic anemia among HIV/AIDS patients attending University of Gondar Hospital, Northwest Ethiopia. In: International Conference on Hematology & Blood Disorders. Journal of Blood Disorders & Transfusion. Hilton Baltimore-BWI Airport, USA; 2014.
40. Kaweme NM, Butress SMN, Mantina HM. Long-Term Zidovudine Therapy and Whether It is a Trigger of Vitamin B12 Deficiency: A Case Study of Megaloblastic Anemia at the University of Zambia Teaching Hospital. Case Rep Hematol. 2022;2022:1-6. doi:10.1155/2022/3827616

