


Hematological Abnormalities of Adult HIV-Infected Patients Before and After Initiation of Highly Active Antiretroviral Treatment at Debre Tabor Comprehensive Specialized Hospital, Northcentral Ethiopia: A Cross-Sectional Study

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Background: Hematological abnormalities have been associated with an increased risk of disease progression and death in people living with human immunodeficiency virus (HIV). The use of antiretroviral medications can have a positive or negative effect on the hematological disorder. However, little is known about its impact on hematological parameters in antiretroviral-treated patients in Ethiopia, especially in the study area.

Methods: A cross-sectional study was conducted at Debre Tabor Comprehensive Specialized Hospital from September to November 2020. A total of 334 HIV-infected patients taking highly active antiretroviral treatment (HAART) at least for 6 months were selected using a simple random sampling technique. Socio-demographic and clinical characteristics of the study subjects were collected using a semi-structured questionnaire. Hematological and immunological parameters were determined using Sysmex kx-21 hematology analyzer and BD FACS count CD4 analyzer, respectively. Statistical analysis was done using SPSS version 20 statistical software. A P-value <0.05 was considered statistically significant.

Results: A total of 334 HIV patients were included in this study. The prevalence of anemia, leucopenia, neutropenia, lymphopenia and thrombocytopenia were 37.1%, 22.8%, 8.4%, 10.5% and 17.1% before initiation of HAART and 17.4%, 34.2%, 18.8%, 13.1% and 8.3% after initiation of HAART, respectively. There was a significant difference in total white blood cell (WBC) count, absolute neutrophil count (ANC), red blood cell (RBC) count, hemoglobin value, mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), red cell distribution width (RDW), platelet and CD4+ T cell counts in HIV patients before and after initiation of HAART (P<0.05).

Conclusion: The most common hematological abnormalities observed in this study before and after HAART initiation were anemia, leucopenia, neutropenia, lymphopenia, and thrombocytopenia. However, after beginning HAART, the prevalence of anemia and thrombocytopenia decreased dramatically.

Keywords: HIV, anemia, leucopenia, thrombocytopenia, HAART

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Introduction

The most common complication of human immunodeficiency virus (HIV) infection is hematological abnormalities. As the disease progresses, these abnormalities become more pronounced.¹⁻³ These abnormalities are the major causes of

morbidity and mortality in HIV patients.⁴ Abnormalities of the bone marrow occur at all stages of HIV infection.^{1,3}

Anemia, neutropenia, lymphopenia, and thrombocytopenia are hematologic complications of HIV infection.⁵ Anemia is the most common hematological disorder that frequently occurs in HIV patients and it is an indicator of transition to acquired immune deficiency syndrome (AIDS) or death. More than 70% of people living with HIV got anemia.^{6,7} Anemia is associated with an increased risk of disease progression and death in both antiretroviral-treated and untreated individuals.⁴ HIV-related anemia can be caused by a variety of factors, including opportunistic infections, chemotherapeutic treatment side effects, changes in cytokine expression leading to a decline in blood cell development, HIV-related metabolic disorders, and micronutrient deficiencies.^{8–10}

Thrombocytopenia is a common complication of HIV infection.¹¹ It affects 4.1–40% of HIV patients, and the prevalence and severity of the disorder increase as the disease progresses.^{12,13} Increased platelet destruction caused by nonspecific deposition of circulating immune complexes on platelets or the presence of specific antiplatelet antibodies, as well as direct infection of megakaryocytes by HIV with a resulting decrease in platelet production, have all been reported as possible mechanisms for the occurrence of thrombocytopenia in HIV patients.^{11,14}

Another abnormality that occurs in HIV patients is leucopenia, which is a reduction in white blood cell (WBC) count. Neutropenia is the most common leucopenia, occurring in 5–30% of patients with early symptomatic HIV infection and up to 70% of patients with advanced stages of AIDS.^{13,15} HIV-associated neutropenia is caused by several causes, including HIV infection itself, autoimmune disorders, opportunistic infections, and medications used to treat HIV and opportunistic infections.^{16,17} Furthermore, as HIV infection progresses, lymphopenia develops, resulting in a decrease in the cluster of differentiation 4 (CD4+) T-cell lymphocytes.¹⁸

Depending on the antiretroviral drug formulation used, antiretroviral therapy can have a positive or negative effect on hematological parameters. While many medications used to treat HIV-related disorders are myelosuppressive, the use of zidovudine is the most common cause of severe cytopenia.^{19,20}

In resource-constrained countries like Ethiopia, hematological abnormalities in HIV patients continue to be a concern. The detection of hematological defects during

antiretroviral therapy provides the best possible care for HIV patients. Even though different studies were conducted to assess the hematological abnormalities among HIV-infected individuals, data are scarce on the severity of hematological abnormalities in HIV patients before and after HAART initiation in our study area. Thus, the purpose of this study was to assess hematological abnormalities in HIV-positive adults before and after starting HAART at Debre Tabor Comprehensive Specialized Hospital in north-central Ethiopia.

Materials and Methods

Study Area

The study was conducted at the ART clinic of Debre Tabor Comprehensive Specialized Hospital. The hospital is located 666km away from Addis Ababa, the capital city of Ethiopia, in north-central Ethiopia. Hospital is organized in 1923 E.C and it is one of the biggest hospitals in the Amhara region that provides health services for over 1.7 million inhabitants in the area. The hospital started delivering ART service in 2006 and currently providing service for more than 2150 HIV patients.

Study Design, Period and Subjects

An institution-based cross-sectional study was conducted from September 1 to November 30, 2020, to assess hematological abnormalities of adult HIV-infected Patients before and after initiation of HAART at the ART clinic of Debre Tabor Comprehensive Specialized Hospital. All HIV-positive patients who visited the ART clinic during the study period and those who fulfilled the eligibility criteria were included in the study.

Eligibility Criteria

Adults (≥ 18 years old) HIV patients at the time of ART initiation, patients having complete hematological value at the baseline, those who were on ART for at least six months, and those who were volunteered to participate in the study were included in the study. Those who were severely sick due to other medical conditions, those who were on medication, those who were diagnosed as having hematological diseases, and pregnant were excluded from the study.

Sample Size and Sampling Technique

The sample size was calculated using a single population proportional formula by considering the following

assumption: by using prevalence of leucopenia as 35.9% from the previous study,¹⁰ marginal error of 5%, level of confidence 95% and since the source population was <10,000, correction formula was applied. Using the above assumption, the calculated minimum sample size was 304 and adding 10% non-response rate making the final sample size to be 334. The study participants were selected using simple random sampling technique.

Data Collection and Laboratory Method

Socio-demographic characteristics and clinical data were collected by trained nurses using a pre-tested semi-structured questionnaire. The data were collected by interview and review of the medical record. The baseline data were collected from the patients' medical charts. Trained laboratory technologists collected a volume of 3–5 mL EDTA anticoagulated whole blood from each study participant and performed the laboratory analysis. Hematological parameters were determined using Sysmex kx-21 hematology analyzer (Sysmex Corporation, China), and CD4+ T cells were determined using the BD FACSCOUNT system (Becton Dickinson and Company, California, USA). The manufacturer instructions were strictly followed for each parameter.

Data Quality Assurance

The 5% of the questionnaire was pre-tested in Estie primary hospital, which is found outside the study area, for its accuracy and consistency before actual data collection. The one-day training was given for data collectors about the objective and relevance of the study, confidentiality, study participants' right, consenting, techniques of interview, and regarding laboratory test procedures and their quality control. Socio-demographic and clinical data were collected by four trained BSc nurses under the supervision of investigators. The laboratory test was performed by two senior medical laboratory technologists. Furthermore, the investigators closely follow up and frequently checked the data collection process to ensure the completeness and consistency of the collected data and also gave feedback on daily basis to the data collectors. The completion, accuracy, and clarity of the collected data were checked carefully.

All laboratory tests were analyzed after the quality control sample ran and the method ensured to be safe. For Sysmex kx-21 hematology analyzer, EIGHTCHECK-3WP X-TRA levels (low, normal, high) commercial control were run daily to monitor the performance of the

system. For BD FACSCOUNT, BD FACSCount control (Zero, Low, Medium, and High level) was run daily for checking linearity and to monitor the performance of the machine. The manufacturer instructions were strictly followed for each quality control reagent. Pre-analytical, analytical, and post-analytical stages of quality assurance were strictly followed by using laboratory manuals and standard operating procedures (SOPs) of Debre Tabor Comprehensive specialized hospital laboratory.

Data Analysis and Interpretation

The coded data were cleaned, edited, checked for completeness, and then entered into Epi Info version 7 and transported to SPSS version 20 statistical software. Categorical variables were summarized in frequency and percentages. Continuous variables were presented in mean and standard deviation. Paired *T*-test was used to compare the means of each hematological parameter before and after 6 months of HAART initiation. A *p*-value <0.05 was considered as statistically significant.

Operational Definitions

Anemia was defined as Hgb concentration <130g/l for adult males and <120g/l for adult females. Leucopenia was defined as a total WBC count less than 4×10^9 cells/l, whereas thrombocytopenia was defined as platelet count $<150 \times 10^9$ /l. Neutropenia was defined as absolute neutrophil count $<1 \times 10^9$ cells/l, whereas Lymphopenia was defined as absolute lymphocyte count $<0.8 \times 10^9$ cells/l.^{1,3}

Ethical Consideration

Ethical clearance was obtained from the Research and Ethical Review Committee of the College of Health Sciences, the letter's reference number was CHS/1427/2020, Debre Tabor University. Then, permission was taken from Debre Tabor hospital higher management. The study was conducted in accordance with the Declaration of Helsinki. The data were collected after obtaining written informed consent from both literate participants and the legal guardians of all illiterate participants. There was no financial compensation or provision for the study participants. To ensure confidentiality of data, the study participants were identified using codes, and unauthorized persons had no access to the collected data. Furthermore, all findings were utilized for the proper management of the patients.

Result

General Characteristics of the Study

Participants

A total of 334 HIV-infected individuals were included in the study. Their ages ranged from 20 to 67 years with a mean age of 38.8 ± 9.9 years and a median age of 37 years. The majority 117 (35%) of the age groups were between 40 years to 49 years. Out of the 334 patients, 212 (63.5%) were females and 122 (36.5%) were males. The majority of study participants 232 (69.4%) were married. About 135 (40.4%) of the study participants were under WHO clinical stage III. The most widely used ART regimen 210 (62.8%) in this study was 1e (TDF-3TC-EFV) (Table 1).

Hematological and Immunological Values Before and After Initiation of HAART

There were statistically significant differences in the mean values of total white blood cell (WBC) count ($6.5 \pm 2.4 \times 10^9/l$ vs. $5.1 \pm 1.2 \times 10^9/l$), absolute neutrophil count (ANC) ($3.1 \pm 1.8 \times 10^9/l$ vs. $1.9 \pm 1.6 \times 10^9/l$), red blood cell (RBC) count ($4.56 \pm 0.76 \times 10^{12}/l$ vs. $4.14 \pm 0.6 \times 10^{12}/l$), hemoglobin (Hgb) value (129 ± 20.5 g/l vs 141.7 ± 17.5 g/l), mean cell volume (MCV) (87.17 ± 3.51 fl vs 100.67 ± 4.9 fl), mean cell hemoglobin (MCH) (28.75 ± 3.19 pg vs 34.04 ± 4.15 pg), mean cell hemoglobin concentration (MCHC) (328.6 ± 20.3 g/l vs 338.9 ± 18.3 g/l), red cell distribution width (RDW) ($14.85 \pm 2.18\%$ vs $13.03 \pm 1.5\%$), platelet (PLT) count ($268 \pm 85.2 \times 10^9/l$ vs. $300 \pm 83.6 \times 10^9/l$) and cluster of differentiation 4 (CD4)+ T cell counts (161 ± 106.5 cells/mm³ vs 381.2 ± 190.9 cells/mm³) before and after HAART initiation, respectively ($P < 0.05$) (Table 2).

Prevalence of Hematological Abnormality

Hematological abnormalities were observed both before and after treatment with HAART. One of the common hematological abnormalities found in this study was anemia which was before the initiation of HAART 37.12% with (76.7% mild, 22.4% moderate, and 0.9% severe anemia) but after six months of HAART, the prevalence of anemia was reduced to 17.36% with (85.5% mild and 13.5% moderate). The other common abnormality found in this study were Leucopenia, Neutropenia, Lymphopenia, and thrombocytopenia. Except anemia and

Table 1 Characteristics of HIV-Positive Adult Individuals at Debre Tabor Comprehensive Specialized Hospital, Debre Tabor, Northcentral Ethiopia, 2020

Variables		Frequency (n=334)	Percentage (%)
Age (in years)	20–29	59	17.6
	30–39	122	33.5
	40–49	117	35
	50–59	27	8.1
	60–69	9	2.7
Sex	Male	122	36.5
	Female	212	63.5
Residence	Urban	243	72.8
	Rural	91	27.2
Religion	Orthodox	303	91
	Muslim	9	2.4
	Protestant	22	6.6
Marital Status	Single	89	26.6
	Married	232	69.4
	Divorced	6	1.8
	Windowed	7	2.2
Educational status	Illiterate	106	31.7
	Primary school	121	36.2
	Secondary school	56	16.8
	Certificate and above	51	15.3
Occupational status	Employed in public organization	40	11.9
	Employed in private organization	37	11.1
	Self employed	179	53.6
	Un employed	78	23.4
WHO clinical stages at the base line	Stage I	56	16.8
	Stage II	62	18.6
	Stage III	135	40.4
	Stage IV	81	20.2
Type of ART regimens	1c	29	8.7
	1d	46	13.8
	1e	210	62.8
	1f	49	14.7
Cotrimoxazole prophylaxis	Yes	72	21.6
	No	262	78.4

Abbreviations: WHO, World Health Organization; 1c, AZT-3TC-NVP; 1d, AZT-3TC-EFV; 1e, TDF-3TC-EFV; 1f, TDF-3TC-NV.

Table 2 Hematological and Immunological Parameters of HIV-Positive Adult Individuals at Debre Tabor Comprehensive Specialized Hospital, Debre Tabor, Northcentral Ethiopia, 2020

Parameters	Before Initiation of HAART (n= 334) Mean ± SD	After Six Months of HAART Initiation (n= 334) Mean ± SD	P-value
WBC ($\times 10^9/l$)	6.5±2.4	5.1±1.2	<0.001*
TLC ($\times 10^9/l$)	2.5± 0.8	2.3±0.7	0.376**
ANC ($\times 10^9/l$)	3.1±1.8	1.9±1.6	<0.001*
RBC ($\times 10^{12}/l$)	4.56±0.76	4.14±0.6	<0.001*
Hgb (g/l)	129±20.5	141.7±17.5	<0.001*
MCV (fl)	87.17±3.51	100.67±4.9	<0.001*
MCH (pg)	28.75±3.19	34.04±4.15	<0.001*
MCHC (g/l)	328.6±20.3	338.9±18.3	<0.001*
RDW (%)	14.85±2.18	13.03±1.5	<0.001*
PLT ($\times 10^9/l$)	268±85.2	300±83.6	<0.001*
CD4 (Cells/mm ³)	161±106.5	381.2±190.9	<0.001*

Notes: *P<0.05 statistically significant association, **P>0.05 statistically non-significant association.

Abbreviations: WBC, white blood cell; TLC, total lymphocyte; ANC, absolute neutrophil count; RBC, red blood cell; Hgb, hemoglobin; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; RDW, red cell distribution width, PLT, platelets; CD, cluster of differentiation.

thrombocytopenia, the other abnormalities were increased after initiation of HAART (Table 3).

Discussions

This study investigates the hematological abnormalities of 334 HIV-positive adult individuals before and after taking HAART for more than six months. Thus, the commonest hematological abnormalities found in the current study were anemia, leucopenia, neutropenia, lymphopenia, and thrombocytopenia. In the present study, prevalence of anemia and thrombocytopenia decreased and the prevalence of Leucopenia, Neutropenia, and Lymphopenia was an increase after initiation of HAART as observed from other studies.^{10,21}

The prevalence of anemia in the present study was 37.1% at the baseline. This finding is in line with a study done in Europe and North America that reported that the prevalence of anemia at the baseline was 35%.²² After 6

months of HAART the prevalence of anemia in the current study was 17.4%. This finding is consistent with the study done in South West Ethiopia which reported that the prevalence after 6 months of HAART was 16.2%.²³ The reduction of anemia after HAART initiation indicates the efficacy of the HAART in the reduction of the viral load which contributed to the differentiation and survival of blood cells. Therefore, HIV patients who were on HAART had greater numbers of blood cells within six months of beginning treatment.²⁴

The current study had a different prevalence of anemia before and after HAART initiation when compared to other studies. A study from Gondar, Ethiopia reported the prevalence of anemia as 29.7% before HAART and 11.7% after HAART.¹⁰ A study from Addis Ababa, Ethiopia reported the prevalence as 41.9% and 11.4% before and after HAART initiation, respectively.²⁵ Other studies from Addis Ababa, Ethiopia reported the prevalence as 24.1% before HAART and 11.98% after HAART initiation.²⁶ The study from Nigeria reported that the prevalence of anemia was 57.5% and 24.3% before and after HAART, respectively.²¹ Moreover, the study from Ghana reported that 63% before HAART and 46% after HAART.²⁷ This difference might be because of the different factors such as the difference in the study population, sample size, study design, socio-demographic characteristics of study subjects, and variability in the definition of anemia.

In the present study, after initiation of HAART the mean value of hemoglobin level and Red cell indices (MCV, MCH, and MCHC) had significantly increase

Table 3 Hematological Abnormalities in HIV Patients at Debre Tabor Comprehensive Specialized Hospital, Debre Tabor, Northcentral Ethiopia, 2020

Hematological Disorder	Before Initiation of HAART	After 6 Months of Initiation of HAART
Anemia	124 (37.1%)	58 (17.4%)
Leucopenia	76 (22.8%)	114 (34.2%)
Neutropenia	28 (8.4%)	63 (18.8%)
Lymphopenia	35 (10.5%)	44 (13.1%)
Thrombocytopenia	57 (17.1%)	28 (8.3%)

whereas the mean value of RBC and RDW had significantly decreased ($p < 0.001$). This finding is consistent with other studies.^{10,25,28}

The prevalence of Leucopenia in the current study was 22.8% and 34.2% before HAART and after 6 months of HAART initiation, respectively. The prevalence of leukopenia after HAART (34.2%) agrees with the study from Gondar, Ethiopia reported that the prevalence of Leukopenia after HAART is 35.9%.¹⁰ However, the current study had a different prevalence of leucopenia before and after HAART when compared with the study from Nigeria which reported that the prevalence of leucopenia was 6.1% and 1.7% before and after HAART, respectively.²¹ This difference might be due to the difference in the study population, sample size, study design, and clinical condition.

The prevalence of Neutropenia in the present study was 8.4% and 18.8% before and after 6 months of HAART initiation, respectively. This finding varies from the study reported from Gondar, Ethiopia that reports neutropenia as 14.8% and 28.3% before and after initiation.¹⁰ This discrepancy might be because of variation in the study population, sample size, study design, and clinical condition.

The prevalence of thrombocytopenia in the present study was 17.1% and 8.3% before HAART and after 6 months of HAART initiation, respectively. The prevalence of thrombocytopenia in the current study is high before initiation of HAART when compared to patients on HAART like other studies.^{10,21,29} The increased prevalence of thrombocytopenia before initiation of HAART might be due to immune destruction of the platelet.³⁰ In the current study, the prevalence of thrombocytopenia before initiation of HAART is similar to Uganda and Nigeria studies that reported as 17.8% and 16.1% before initiation of HAART, respectively.^{8,29} However, the present finding higher than the study report from Gondar, Ethiopia that reports 9% and 4.1%, and the study from other Nigeria which reported as 9.6% and 1.2% before and after initiation of HAART.^{10,21} This difference might be because of the difference in the study population, sample size, study design, and clinical condition.

This study had the limitation. The study did not address viral load measurement because of a lack of resources. Besides, the study focused only on the hematological abnormality but it did not address the risk factors.

Conclusion

Anemia, leucopenia, neutropenia, lymphopenia, and thrombocytopenia were found to be the most common

hematologic abnormalities in HIV/AIDS patients. Total WBC count, neutrophil, lymphocyte, Hgb, MCV, MCH, MCHC, and platelets had statistically significant differences before and after HAART initiation. Additionally, large-scale and longitudinal studies are recommended to strengthen and explore the problem in depth.

Abbreviations

AIDS, acquired immune deficiency syndrome; ART, antiretroviral therapy; ANC, absolute neutrophil count; AZT, azidothymidine; CBC, complete blood cell count; CD4, cluster of differentiation 4; HAART, highly active antiretroviral therapy; Hgb, hemoglobin; HCT, hematocrit; HIV, human immune deficiency virus; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; MCV, mean cell volume; PLT, platelets; RBC, red blood cell; RDW, red cell distribution width; TLC, total leukocyte count; WBC, white blood cells; WHO, World Health Organization.

Data Sharing Statement

All data generated or analyzed during this study are included in this manuscript.

Ethics Approval and Informed Consent

Ethical clearance was obtained from the Research and Ethical Review Committee of the College of Health Sciences, the letter's reference number was CHS/1427/2020, Debre Tabor University. Then, permission was taken from Debre Tabor hospital higher management. The study was conducted in accordance with the Declaration of Helsinki. After obtaining written informed consent from all literate participants and the legal guardians of all illiterate participants, the data was collected. There was no monetary reward or provision for the participants in the research. To ensure confidentiality of data, the study participants were identified using codes, and unauthorized persons had no access to the collected data. Furthermore, all findings were utilized for the proper management of the patients.

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Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, analysis, and interpretation of data; took part in drafting the article, revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work.

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