





Prevalence of Leucopenia and Associated Factors before and after Initiation of ART among HIV-Infected Patients, North East Ethiopia: Cross-Sectional Study

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Background: Leucopenia is the commonest hematological abnormality that occurs in patients with human immune deficiency virus (HIV) infection. The magnitude and related factors of leucopenia during the time of ART are not characterized in Ethiopia. This study aimed to assess the prevalence of leucopenia before and after the initiation of ART among HIV patients attending Debre Berhan Referral Hospital (DBRH), North East Ethiopia.

Methods: A cross-sectional study was conducted from September to December 2020 in DBRH, North-East Ethiopia. A total of 272 patients on ART were selected by simple random sampling techniques. Socio-demographic and clinical characteristics of the study participants were collected by standard questionnaires. Measurements of leucocyte count and CD4 counts were made by Sysmex XT 2000i hematology analyzer and BD FACS count CD4 analyzer, respectively. Statistical analysis of data was done by SPSS version 23. Logistic regression was done and a P -value < 0.05 was taken as statistically significant.

Results: The prevalence of leucopenia, neutropenia, and lymphopenia were 20.9%, 7.0%, and 6.6% before initiation of ART and 15.4%, 1.1, and 4.4% after initiation of ART, respectively. There was a significant difference in total white blood cell count, absolute neutrophil count, and total lymphocyte count between patients on ART and ART naïve patients. HIV patients whose cluster of differentiation (CD4) counts were < 200 cells/μL and patients on a zidovudine (AZT)-based regimen were more likely to have leucopenia than HIV patients whose CD4 counts were \geq 200 cells/μL and on a tenofovir (TDF)-based regimen.

Conclusion: In this study, the prevalence of leucopenia, neutropenia, and lymphopenia has shown a significant decrement after the initiation of ART. HIV patients with low CD4 count and on an AZT-based regimen are more likely to have leucopenia, neutropenia, and lymphopenia. Based on our findings, we recommend that the health care professional routinely investigate and should treat leucopenia.

Keywords: ART, leucopenia, HIV/AIDS, Ethiopia

Background

Hematologic disorders are the most common complications of HIV AIDS, which include anemia, leucopenia, and thrombocytopenia.¹ Among these hematological abnormalities; leucopenia is the common hematological abnormality that occurs in patients with HIV infection. The cause of HIV-related leucopenia is multifactorial, which includes a direct effect of HIV, autoimmune disease, neoplasm and ART

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drugs (especially zidovudine-based regimen), cotrimoxazole, and opportunistic infection.²⁻⁴ The prevalence of leucopenia is higher among treatment naïve people than treated ones in most studies.³⁻⁵ The use of antiretroviral therapy (ART) improves total white blood cell count by preventing suppression of bone marrow cells from the HIV virus and it blocks the interference of virus in the hematopoiesis process.⁶ Leucopenia occurs in HIV-infected patients. Even though low leukocyte count mainly indicates the toxicities of drugs for HIV or associated conditions, studies of untreated patients have also shown a high magnitude of leucopenia.⁷ The most common form of leucopenia usually encountered is neutropenia, occurring in 10–30% of HIV-infected individuals.⁸ Neutropenia is caused by decreased production of cells because HIV suppresses the bone marrow by changing the marrow microenvironment and by altering the cytokine expression and it is also common among HIV-infected patients and presents in approximately 10–50% of cases.^{8,9} In the study conducted in different parts of the United States, worsening of HIV disease parameters, like lower CD4 counts and higher HIV viral load, was found to be associated with the development of neutropenia.¹⁰ ART showed a statistically significant increment in the mean WBC (White blood cells) count in different studies.¹¹⁻¹⁵ On the other hand, some studies reported that patients on ART had low WBC count compared to treatment naïve patients.^{16,17} Patients on ART showed a significant reduction in the prevalence of leucopenia and neutropenia compared to their ART-naïve groups.^{6,18} The improvement in these hematological abnormalities after ART initiation was due to increased production of Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) and Granulocyte Colony Stimulating Factor (G-CSF), which play an important role in the activation of granulocytes.¹⁹ Studies indicated that low CD4 count, advanced clinical stage of HIV disease, and zidovudine-based regimen are associated with an increased risk of leucopenia.^{8,16,20-22} Although hematologic abnormalities have been widely reported in HIV-related infections, there are a few data related with the prevalence of leucopenia and associated factors of leucopenia among HIV-infected patients before and after initiation of ART in Ethiopia. This study provides further information on HIV-associated leucopenia, and it can serve as a baseline for future studies. The aim of this study is to determine the prevalence of leucopenia and associated factors before and after the initiation of ART

among HIV patients who attended the ART clinic at DBRH, North-East Ethiopia.

Methods and Materials

Study Period and Area

This study was conducted at the ART clinic of DBRH, North East Ethiopia from September to December 2020.

Study Design

A hospital-based cross-sectional study was conducted.

Source Population

All adult HIV-1 infected people receiving ART at the DBRH.

Study Population

All adult HIV-1 infected people receiving ART for at least 6 months in the ART clinic of DBRH.

Inclusion and Exclusion Criteria

Age greater or equal to 18-years old HIV-positive individuals who received ART for at least 6 months in DBRH were included in this study. Pregnant women, patients referred from other health institutions, patients with hematological disorders, severely sick patients with any other medical conditions, and patients who had taken other medications were excluded from the study.

Sample Size Determination

The sample size was determined by using a statistical for a single population ($n = z^2 p (1-p)/d^2$), taking $p=19\%$ (prevalence of leucopenia from previous study) 85% level of precision (d) with 95% confidence interval plus 15% non-response rate were added.

$n = (z^2 p (1-p)/d^2)$, substitute the value, $n = 1.962^2 * 0.19 * 0.81 / 0.052^2 = 236.48$, when we added a 15% non-response rate we found approximately 272 sample sizes.

Sampling Procedures and Techniques

The samples of this study were collected by simple random sampling technique until the desired sample size was achieved from the subjects who fulfilled the inclusion criteria during the study period.

Study Variables

Dependent Variables

Prevalence of leucopenia, neutropenia, and lymphopenia

Independent Variables

Age, sex, stage of HIV disease, CD4 count, and type of ART regimen.

Data Collection and Procedures

The standard structured questionnaire was adapted after the review of different literature, and the data was collected by trained ART nurses. Data about socio-demographic, clinical characteristics, and pre-ART information of the study participants were collected by face-to-face interview and a review of medical registration books. After that, a blood sample was collected by laboratory technicians and sent to a hematology analyzer. Based on the standard procedures, WBC count and CD4 cell counts were determined by using the Sysmex XT 2000i hematology analyzer and BD FACS count system, respectively. In order to keep the quality of data, standard procedures were followed in every laboratory aspect of the procedures and the quality of CD4 and hematology analyzer was checked by running quality control samples along with the patient sample. In addition, there was training for data collectors, a pretest of the questioner was made and the data collection process was done two times a day by the principal investigators.

Leucopenia: Defined as WBC counts <4,000 cells/ μ L.

Neutropenia: Absolute neutrophil count (ANC) less than 1,000 cells/ μ L.

Lymphopenia: Total lymphocyte count (TLC) of less than 800 cells/ μ L.

Data Processing and Analysis

The data were coded, checked and entered in the EPI data software version 3.1, and exported to SPSS software version 23 for analysis. The results of descriptive statistics were expressed as frequency and percentage. Continuous variables were presented as mean \pm standard deviation. Univariate logistic regression was performed to examine the association between dependent and independent variables using crude odds ratio (COR) with a 95% confidence interval (CI). Those independent variables with a P -value<0.2 in univariate analysis were included in multivariate logistic regression models. A P -value<0.05 on multivariate logistic regression was considered a statistically significant association.

Result

General Characteristics

From the total 272 HIV-positive patients, 110 (40.4%) were male and 162 (59.6%) were female. The mean age

of the study participants was 40.94 ± 10.88 years, ranging from 18–78 years. Most of the study participants were within the WHO stage one category at the baseline.

The WBC Count, ANC, TLC, and CD4 Counts of the Study Participants

The mean WBC count, ANC, and TLC at baseline were $4.65 \pm 1.53 \times 10^3/\mu\text{L}$, $2.24 \pm 0.9 \times 10^3/\mu\text{L}$, $1.48 \pm 0.6 \times 10^3/\mu\text{L}$, and $5.58 \pm 1.82 \times 10^3/\mu\text{L}$, $2.92 \pm 1.38 \times 10^3/\mu\text{L}$, $1.81 \pm 0.72 \times 10^3/\mu\text{L}$ after initiation of ART ($P < 0.01$). Similarly, the mean CD4 counts showed an increment from 264.75 ± 184.5 cells/ μL at the baseline to 544.0 ± 261.3 cells/ μL after the initiation of ART ($P < 0.01$). The most commonly used ART regimen in the current was 1j (TDF-3TC-DTG) (Table 1).

Prevalence of Leucopenia, Neutropenia, and Lymphopenia and Associated Factors before Initiation of ART

The prevalence of leucopenia, neutropenia, and lymphopenia before ART was 20.9%, 7.0%, and 6.6%, respectively. The prevalence of leucopenia in males was 24.5% and 17.9% in females, whereas the prevalence of neutropenia and lymphopenia were 8.2%, 7.3% and 5.6%, 5.6% in males and females, respectively. In this study, most of the leucopenia cases (27.12%) were observed in the age groups ≥ 50 years. However, the majority of neutropenia cases were observed in the age range between 40–49 years and lymphopenia falls between the age group 18–19 years. Patients whose CD4 counts were <200 were strongly associated with a high prevalence of leucopenia and lymphopenia with a P -value<0.05. Lymphopenia was significantly associated with the advanced stage of HIV disease with a P -value<0.03 (Table 2).

Prevalence of Leucopenia, Neutropenia, and Lymphopenia, and Associated Factors after the Initiation of ART

The prevalence of leucopenia, neutropenia, and lymphopenia after ART was 15.4%, 1.1, and 4.4%, respectively. The prevalence of leucopenia, neutropenia, and lymphopenia after the initiation of ART was decreased by 5.5%, 5.9%, and 2.2%, respectively. The prevalence of leucopenia, neutropenia, and lymphopenia in males was 24.5%, 0.9%, and 6.4% and in females 17.9, 1.2, and 3.1, respectively (Table 3). The prevalence of leucopenia was higher (21.6%) among patients whose age group was ≥ 50 years. The prevalence of

Table 1 Socio-Demographic and Clinical Characteristics of HIV Positive Patients Taking ART at DBRH, North East Ethiopia, 2020

Variables	Frequency	Percentage
Age in years		
18–29	34	12.5
30–39	97	35.9
40–49	82	30.0
≥50	59	21.6
Sex		
Male	110	40.4
Female	162	59.6
Marital status		
Single	23	49.8
Divorced	39	27.5
Married	135	8.4
Widowed	75	14.3
Educational status		
Illiterate	74	27.1
Primary school	92	33.7
High school	63	23.1
Certificate and above	44	16.1
WHO clinical stages		
Stage I	144	52.9
Stage II	36	13.2
Stage III	87	31.9
Stage IV	5	1.8
Types of ART regimen		
1c	14	5.1
1d	10	3.7
1e	75	27.6
1j	130	47.8
2f	19	7.0
2h	18	6.6
Not registered	6	2.2

Notes: 1c=AZT-3TC-NVP, 1d=AZT-3TC-EFV, 1e=TDF-3TC-EFV, 1f=TDF-3TC-NVP, 1j=TDF-3TC-DTG, 2f=AZT-3TC-ATV/r, 2h=TDF-3TC-ATV/r

leucopenia, neutropenia, and lymphopenia was high in patients with low CD4 count (<200). However, there was no significant association observed between the low CD4 count (<200) and that of leucopenia, neutropenia, and lymphopenia. HIV patients on an AZT-based regimen are more likely to have leucopenia and lymphopenia than HIV patients on a TDF-based regimen with a significant association at a *P*-value <0.02 and 0.01, respectively (Table 3).

Discussion

It's well documented that hematological abnormalities are common in HIV-infected patients.¹ Leucopenia, for

instance, occurs in patients with HIV infection. The cause of HIV-associated leucopenia is multifactorial, including a direct consequence of HIV infection, autoimmune disorders, malignancies, and drugs used to treat HIV.³ This study revealed that the prevalence of leucopenia, neutropenia, and lymphopenia were 20.9%, 7.0%, and 6.6% at baseline, and 15.4%, 1.1%, and 4.4% after the initiation of ART. A study conducted in Ghana reported that the prevalence of leucopenia, neutropenia, and lymphopenia were 13%, 72.5%, and 6.5% in pre-ART patients and 6.5%, 85%, and 18% in post-ART patients.⁴ Another study conducted in Gondar, Ethiopia reported that the prevalence of leucopenia, neutropenia, and lymphopenia were 35.9%, 28.3, and 2.1 on ART patients and 16.9%, 14.5, and 2.1 ART naïve patients.¹⁶ The difference in results seen from the present study might be due to the difference in the definition of leucopenia, neutropenia, and lymphopenia, study design and size of the study population. The decrease in the prevalence of leucopenia, neutropenia, and lymphopenia after ART initiation might be due to; disorders of hematopoiesis, opportunistic infections, and immune causes related to HIV leading to low white blood cell count could be reverted after ART initiation.²³ In the present study the prevalence of leucopenia was elevated in patients whose age is ≥50 years old, which is in agreement with another study.² The increase in the prevalence of leucopenia with age might be due to a higher incidence of myelodysplasia in older patients.⁹ However, leucopenia did not show a statistically significant difference with sex and age. This was in line with previous studies.^{2,21}

According to the present study, the prevalence of leucopenia, neutropenia, and lymphopenia were increased with decreasing in CD4 count both before and after the initiation of ART. Leucopenia, neutropenia, and lymphopenia were more prevalent among HIV-positive patients whose CD4 count was <200 cells/μL. These findings were similar to several studies that reported the leucopenia, neutropenia, and lymphopenia was more prevalent among patients with CD4 count <200 cells/μL.^{2,3,16} The prevalence of leucopenia and lymphopenia with decreased CD4 count was significantly associated before initiation of ART, but it did not significantly associate after ART initiation, this may be due to ART bringing a statistical increment into the WBC count.^{11,13} The present study revealed that patients on AZT-based ART regimen had a higher prevalence of leucopenia, neutropenia, and lymphopenia compared to TDF-based ART regimen. Similar to the

Table 2 Leucopenia, Neutropenia, and Lymphopenia and Associated Factors before ART Initiation in HIV Positive Patients Attended at DBRH, North East Ethiopia, 2020

Variables	Leucopenia, N (%)	COR (95% CI)	AOR (95% CI)	Neutropenia, N %	COR (95% CI)	AOR (95% CI)	Lymphopenia, N (%)	COR (95% CI)	AOR (95% CI)
Age groups									
18–29	7 (20.6)	1.4 (0.52–3.94)	1.3 (0.42–3.96)	1 (2.9)	3.7 (0.43–32.4)	3.7 (0.42–32.2)	3 (8.8)	0.6 (0.11–2.91)	0.6 (0.09–2.92)
30–39	18 (18.4)	1.7 (0.77–3.57)	1.2 (0.49–2.76)	3 (3.1)	3.6 (0.86–14.92)	5.4 (1.04–27.6)	5 (5.1)	0.9 (0.23–4.33)	0.9 (0.18–4.27)
40–49	16 (19.5)	1.5 (0.69–3.39)	1.1 (0.45–2.65)	9 (11.9)	0.9 (0.31–2.74)	0.9 (0.31–2.87)	7 (8.5)	0.6 (0.14–2.32)	0.4 (0.09–1.87)
≥50	16 (27.1)	1	1	6 (10.2)	1	1	3 (5.1)	1	1
Sex									
Male	27 (24.5)	0.7 (0.37–1.21)	0.9 (0.47–1.8)	9 (8.2)	0.7 (0.25–1.72)	0.8 (0.3–2.1)	8 (7.3)	0.8 (0.28–2.01)	1.1 (0.39–3.3)
Female	9 (17.9)	1	1	10 (5.6)	1	1	10 (5.6)	1	1
Clinical stage									
Stage I	& II	29 (16)	2.3 (1.26–4.16)	1.6 (0.81–3.12)	9 (5.0)	2.3 (0.91–5.96)	2.6 (0.97–6.93)	6 (3.3)	4.4 (1.58–12.07) *
Stage III	& IV	28 (34.4)	1	1	10 (10.9)	1	1	12 (13.0)	1
CD4 count cells/ µL									
<200	48 (41.0)	0.1 (0.02–0.21)*	0.1 (0.02–0.26)**	19 (16.2)	–	–	16 (13.7)	0.1 (0.01–0.68)*	0.1 (0.02–0.9)**
200–349	6 (7.2)	0.6 (0.13–2.28)	0.6 (1.15–2.59)	0 (0.0)	–	–	1 (1.2)	1.1 (0.07–18.54)	1.32 (0.08–22.39)
≥350	3 (4.1)	1	1	0 (0.0)	1	1	1 (1.4)	1	1

Notes: *Indicates statistically significant association by univariate analysis, **Statistically significant association by multivariate analysis.
Abbreviations: COR, crude odds ratio; AOR, adjusted odd ratio; CI, confidence interval.

Table 3 Leucopenia, Neutropenia and Lymphopenia and Associated Factors after Initiation of ART in HIV Positive Patients Attended at DBRH, North East Ethiopia, 2020

Variables	Leucopenia, N (%)	COR (95% CI)	AOR (95% CI)	Neutropenia, N %	COR (95% CI)	AOR (95% CI)	Lymphopenia, N (%)	COR (95% CI)	AOR (95% CI)
Age groups									
18–29	4 (11.8)	1.9 (0.57–6.49)	2.2 (0.62–7.6)	1 (2.9)	–	–	1 (2.9)	1.8 (0.18–17.69)	1.9 (0.17–20.81)
30–39	12 (12.2)	1.83 (0.76–4.39)	1.9 (0.78–4.68)	2 (2.0)	–	–	5 (5.1)	0.9 (0.23–4.33)	1.0 (0.21–4.65)
40–49	14 (17.3)	1.22 (0.52–2.88)	1.1 (0.45–2.62)	0 (0.0)	–	–	3 (3.7)	1.4 (0.27–7.16)	1.3 (0.24–7.34)
≥50	12 (20.3)	1	1	0 (0.0)	–	–	3 (5.1)	1	1
Sex									
Male	17 (15.6)	0.7 (0.37–1.21)	0.9 (0.47–1.8)	1 (0.9)	1.3 (0.12–14.94)	3.1 (0.18–49.86)	7 (6.4)	0.5 (0.14–1.5)	0.6 (0.16–2.06)
Female	25 (15.4)	1	1	2 (1.2)	1	1	5 (3.1)	1	1
ART regimen									
AZT based	13 (30.2)	0.4 (0.16–0.74)*	0.3 (0.14–0.65)**	2 (4.8)	0.1 (0.01–1.02)	0.1 (0.01–1.52)	6 (14.0)	0.2 (0.1–0.56)*	0.2 (0.06–0.7)**
TDF based	29 (13.0)	1	1	1 (0.1)	1	1	6 (2.7)	1	1
CD4 count cells/μL									
<200	3 (23.1)	0.6 (0.17–2.45)	–	1 (8.3)	0.5 (0.01–0.87)	0.1 (0.02–1.52)	2 (15.4)	0.2 (0.03–0.98)	0.3 (0.05–1.72)
200–349	4 (9.5)	1.8 (0.61–5.44)	–	1 (2.4)	0.2 (0.01–3.1)	0.3 (0.14–4.32)	3 (7.1)	0.4 (0.12–1.75)	0.13–2.53)
≥350	35 (16.1)	1	1	1 (0.5)	1	1	7 (3.2)	1	1

Notes: *Indicates statistically significant association by univariate analysis, **Statistically significant association by multivariate analysis.
Abbreviations: COR, crude odds ratio; AOR, adjusted odd ratio; CI, confidence interval.

present study conducted in Ethiopia and Ghana, it revealed an increment in the prevalence of leucopenia, neutropenia, and lymphopenia after initiation of AZT.^{2,4} However, neutropenia did not show a statistically significant difference with the AZT-based ART regimen. The high prevalence of leucopenia, neutropenia, and lymphopenia may be due to the suppression of bone marrow by Zidovudine-based therapy.¹⁶

Conclusion

In this study, the prevalence of leucopenia, neutropenia, and lymphopenia had shown a significant decrement after the initiation of ART. HIV patients with low CD4 count and on AZT-based regimen are more likely to have leucopenia, neutropenia, and lymphopenia. Based on our findings, we recommend that health care professionals routinely investigate and should treat leucopenia.

Abbreviations

AIDS, acquired immunodeficiency syndrome; ANC, absolute neutrophil count; ART, anti-retroviral therapy; AZT, zidovudine; CD4, cluster of differentiation; HIV, human immunodeficiency virus; TLC, total lymphocyte count; TDF, tenofovir.

Data Sharing Statement

The datasets used and analyzed during the present study are available from the corresponding author on reasonable requests.

Ethics Approval and Consent to Participate

This study was conducted after ethical letters obtained from the institute of research ethics and review board (IRB) of college of Medicine, Debre Berhan University, Ethiopia complied with the Declaration of Helsinki. The institute of research and ethics board committee (IRB) had reviewed and looded originality, feasibility, laboratory setting, and ethical aspects of the study. Following thorough discussion the committee approved the research proposal by authors with ethical approval using reference number med/219/2019. Then permission was taken from hospital higher management and data was collected after obtaining informed consent from the study participants. To keep confidentiality, codes were used and unauthorized persons did not have access to the data.

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Author Contributions

All authors had valuable contributions to the conception and design, data collection, analysis of data and interpretation; involved in drafting of the article or revising it critically for important intellectual content; agreed on journal to which the article will be submitted; gave final approval of the version to be published; agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing of interests in this work.

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