Hematological and immunological abnormalities among children receiving highly active antiretroviral therapy at Hawassa University College of Medicine and Health Sciences, Southern Ethiopia

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Keywords: HIV, Hematological, Immunological, HAART, Children, Hawassa, Ethiopia

DOI: https://doi.org/10.21203/rs.3.rs-27469/v1

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Abstract

Background: The human immunodeficiency virus (HIV) epidemic remains a serious challenge and continues to take its roll, on vulnerable populations such as children. Hematological and immunological abnormalities are common complications in children infected with human immunodeficiency virus. They are associated with an increased risk of disease progression and death. Hence; specific diagnosis and determination of these parameters are required for monitoring of treatment to avert disease progression. Therefore, this study was aimed to determine hematological and immunological parameters among HAART-experienced children at Hawassa University comprehensive specialized Hospital.

METHODS

A hospital-based cross-sectional study was conducted among 273 HIV-infected children from July to December 2019. Data were collected using a structured questionnaire that included variables related to sociodemographic characteristics and clinical conditions of the study individuals. Blood samples for hematological and immunological parameters were collected and analyzed using SPSS version 20. P-Value < 0.05 considered statistically significant.

RESULTS

A total of 273 HAART-experienced children were enrolled. Of whom 139 (50.9%) and 134 (49.1%) were females and males respectively. The baseline means hemoglobin level of the study participants was 12mg/dl and 40.7% of children were anemic. A baseline CD4+ T-cell median percentage was 18.4% and increased to 29.2% and the mean hemoglobin level at baseline was 12 mg/dl and increased to 13.1mg/dl after treatment. The prevalence of anemia, thrombocytopenia, Leucopenia, Pancytopenia, and neutropenia was 11.4, 4%, 14.3%, 2.9%, and 18.3%, respectively. All forms of hematological abnormalities were highly prevalent in children with a CD4-cell percentage <15%. It was statistically significant with leucopenia (P=0.02), Leucocytosis (P=0.02), and lymphopenia (P=0.001). Similarly, they were highly prevalent with children who had a viral load greater than 150 viral copies /mm3. It was statistically significant with anemia (P=0.002), Lymphopenia (P=<0.001), and pancytopenia (P=0.001).Prevalence of anemia (25%) and Leucocytosis (18.8%) were high among children of age group <5 years.

CONCLUSION:

Hematologic and immunological abnormalities were common problems among children taking highly active antiretroviral therapy. Therefore, clinicians need to routinely investigate for hematological and immunological changes with appropriate therapeutic interventions for hematological and immunological abnormalities after treatment. Furthermore, large scale and longitudinal studies are recommended to strengthen and explore the problem in-depth
The human immunodeficiency virus (HIV) epidemic remains a serious challenge and continues to take its roll, particularly on vulnerable populations such as children. At the end of 2016 UNAIDS reported that there were approximately 36.7 million people worldwide living with HIV/AIDS, of these, 2.1 million were children < 15 years old and 70% (1.5 million) of them were found in Sub-Saharan Africa (1). In the same year, one million people died from acquired immunodeficiency syndrome (AIDS) related illnesses. The annual rate of new HIV infections globally was believed to be 1.8 million (2). Approximately; 12% of these infections (330,000 cases) occurred in children < 15 years of age annually In Ethiopia, there is a significant pediatric HIV burden with approximately 65,100 infected children, with an estimated 3200 AIDS-related child deaths occurring annually (3).

Acquired Immunodeficiency Syndrome (AIDS) is a systemic disorder caused by HIV and characterized by severe impairment and progressive damage of both cellular and humoral immune responses. Besides immunological complications of HIV disease (4) hematological abnormalities have been documented as strong independent predictors of morbidity and mortality in HIV-infected individuals (5) HIV replicates not only in CD4 lymphocyte cells but also in macrophages and dendritic cells (6, 7) such replication is followed by immune system depression, which can lead to life-threatening opportunistic infections. Hematological complications such as mild-to-severe anemia are associated with HIV disease progression and subsequently reduced survival (8).

Although numerous complications occur in HIV infected patients, (5, 9) the most common hematological abnormalities are anemia and neutropenia (10). Anemia and neutropenia are generally caused by inadequate blood cell production because of bone marrow suppression by an HIV infection mediated by abnormal cytokine expression and alteration of the bone marrow microenvironment (8). Anemia in HIV-infected persons is associated with CD4 cell depletion and progression to AIDS (9) and is one of the strongest predictors of HIV mortality and poor responses to antiretroviral therapy (5) Neutropenia is frequently observed in advanced stages of HIV infection after the development of AIDS and has been associated with certain types of antiretroviral medications used to treat HIV infection (11). Thrombocytopenia is characterized by platelet counts below 125 × 103/mm3, and also frequently occurs in HIV-infected patients (12).

The initiation of highly active antiretroviral therapy played a critical role in the clinical management of HIV infected individuals by restoring the immune function, preventing morbidity and mortality, improving quality of life, and preventing the transmission of the virus to other uninfected individuals (13). But a significant number of patients fail to achieve a sustained virological and immunological suppression to treatment among the HIV-infected patients receiving HAART (14). Even after 6–12 months of first-line treatment, several children are failed to attain viral suppression and it could be because these children may have been infected with mutant resistant viruses from mothers that were already on treatment with first-line HAART (15).

Hematological parameters are other important monitoring tools for assessing treatment and prognosis among HAART experienced HIV positive children. The use of antiretroviral drugs could positively or
negatively affect these parameters, depending on the choice of combination of drugs used making hematological abnormalities common among HAART experienced HIV positive persons. For example, lamivudine and zidovudine combination may cause Neutropenia, anemia, thrombocytopenia, and transient rise in liver enzymes, while Nevirapine has been reported as eosinophilia, granulopenia, and jaundice. Anaemia, neutropenia, thrombocytopenia have also been reported as adverse effects of Stavudine (16). Those attributed to Zidovudine and Stavudine include neutropenia, anemia, and thrombocytopenia. Although many drugs used for the treatment of HIV-related disorders are myelosuppressive, severe cytopenias is most often related to the use of zidovudine(17).

Hematological complications have been documented to be the second most common cause of morbidity and mortality in HIV positive children on HAART and are generally marked with cytopenias such as anemia, neutropenia, lymphopenia, and thrombocytopenia(18). The incidence and severity of the cytopenia generally correlate to the stage of the disease with anemia being the most commonly encountered hematologic abnormality and a significant predictor of progression to AIDS or death.

There is limited data regarding the hematological complication, immunologic failure and associated factors among HAART experienced children in the southern region of the country. Therefore, this study was aimed to assess the prevalence of hematological and immunological abnormalities among children on HAART in HUCSH ART pediatrics clinic, Hawassa, southern Ethiopia.

**Methods**

**Study design**

A hospital-based cross-sectional study was conducted from July to December 2019 on 273 HIV-infected children receiving HAART.

**Study area**

The study was done from July to December 2019 at Hawassa University comprehensive specialized Hospital. It is one of the tertiary hospitals in the country with a catchment area of 15–22 million people and the largest academic institutions in Ethiopia. The university is located at the heart of Hawassa city, which is the capital city of the region and located 275 Km from Addis Ababa, the capital city of Ethiopia. The altitude of the city is 1697 meters above sea level with the mean annual temperature and rainfall of 20.9°C and 997.6 mm respectively. The HIV follow-up clinic is scheduled daily, both in the morning and afternoon. The average patient census returning for follow-up was 15–20 patients per day. The hospital started delivering ART service in 2005; currently, there are 273 HIV-positive children enrolled in chronic follow-up care.

**Participants.**

All 273 HIV-infected children < 15 years of age who took HAART for > 6 months who had a follow-up on pediatrics ART clinic at Hawassa University Comprehensive and specialized hospital and who were
voluntary to participate, in the study were included.

Children on treatment for the known hematological disorder and who had transfusion treatment within three months of data collection, those who had a traumatic injury or surgical interventions resulting in blood loss during the study period or within 3 months before the study period and children with their incomplete information, unreadable or their manual record is lost, and who do not have baseline CD4 count and those without a legal guardian or unaccompanied children were excluded from the study.

**Sample size**

To have a representative sample of our entire pediatrics ART follow-up clinic, all 273 children who were on follow up in ART clinic of Hawassa University College of Medicine and Health Science Comprehensive and Specialized Hospital were used as a sample size.

**Data collection tools and procedure**

Data on the sociodemographic and clinical characteristics of the study participants were collected using a pretested structured questionnaire by interview and review of medical records these were composed of sociodemographic characteristics, and for baseline characteristics of the study subjects before initiation of HAART; these included mainly clinical, laboratory and immunological characteristics after initiation of HAART. Data were collected by two trained professional nurses and one pediatrics resident working at the pediatrics ART clinic.

The laboratory measurements were done by experienced laboratory technologists. The data collection process was supervised strictly by the investigator. All study subjects were approached during their respective appointment.

**Laboratory Testing**

**Specimen Collection and Processing**

5 ml of blood was collected by following standard operational procedure (SOP) for sample collection and transport to determine both CBC, CD4 tests, and viral load is drawn from each participant using vacutainer tube containing anticoagulant ethylene diamine tetra-acetic acid (EDTA). Hematological parameters were run on hematological auto-analyzer ruby Cell-Dyne 3000 USA (Abbott Laboratories Diagnostics Division, USA) whereas the immunological (CD4 + T cells) was run on BD FACSCOUNT system (Becton Dickenson and Company, California, USA). The performance of both analyzers was controlled by running quality control material alongside the study participant’s sample. Besides, all flagged specimen was subjected to the manual differential to confirm the results. Immunosuppression and anemia were defined based on WHO criteria (Reference interval) as defined operationally.

**Quality Assurance**
To ensure the quality of data, training was given to data collectors and supervisors, and a pre-test was done on adult patients. The necessary feedback was offered to data collectors in the next morning. Besides, the collected data were checked for validity, completeness, and internal consistency by the principal investigator daily. Quality Control of both CBC and CD4 analyzer was run daily before patient sample analysis.

Data management and analysis

The data were cleaned, edited, checked for completeness, and entered and analyzed using SPSS version 20 statistical software. The results were reported as the mean and standard deviation for continuous variables and as percentages for categorical variables. An unpaired t-test was used to compare the means of all continuous variables. Categorical data were analyzed using the Fisher exact test. Logistic regression analysis was applied to determine the associations of established risk factors for hematologic abnormalities and to determine the determinant of immunologic failure. We used a CI of 95% and $P < 0.05$ to evaluate for any significant association.

Operational definition

Clinical failure

is defined as the appearance or reappearance of WHO clinical stage 3 or stage 4 events after at least 24 weeks on ART in a treatment-adherent child (19).

Immunological failure and anemia: is defined as developing or returning to the following age-related immunological thresholds after at least 24 weeks on ART, in a treatment-adherent child and anemia (19). CD4 count of $< 200$ cells/µL or percent CD4 cell count $< 10\%$ for a child $\geq 1$ year to $< 5$ years of age and a CD4 count of $< 100$ cells/µL for a child 5 years of age or older. Anemia was defined based on WHO criteria (R) as mild anemia: hemoglobin level between 10 and 10.9 g/dL for under 5 and between 11 and 11.9 g/dL for under 18 years of age children; moderate anemia: hemoglobin level between 7.0 and 9.9 g/dL for under 5 and between 8.0 and 10.9 g/dL for under 18 years of age children; severe anemia: hemoglobin level $< 7.0$ g/dL for both under five and under 18 years of age children.

Virological failure

is defined as a persistent HIV viral load of $\geq 5,000$ copies/ml, after at least 24 weeks on ART, in a treatment-adherent child (20).

Based on the definitions of treatment failure above, ART treatment is deemed effective if none of the clinical, immunological, or virological criteria for failure are met after at least 24 weeks on ART. However, for this study, the definition of effectiveness is based on the immunologic and virological criteria for failure. Therefore treatment effectiveness was defined as HIV viral load $< 5,000$ copies/ml after at least 24 weeks on HAART.
**Thrombocytopenia**: defined as platelet count < 150,000/mm³. **Thrombocytosis**: defined as platelet count > 450,000/mm³.

**Leukopenia**

defined as white blood cell (WBC) count < 4000/mm³

**Leucocytosis**

defined as WBC count > 12,000/mm³.

**Lymphopenia**

defined as lymphocyte count < 1500/mm³

**Neutropenia**: defined as absolute neutrophil count (ANC) of < 1500/mm³. Severity further classified as follows: Grade 1: 750–1500/mm³; mild Neutropenia, Grade 2: 500–750/mm³; moderate Neutropenia, Grade 3: <500/mm³; severe Neutropenia, Grade 4: <250/mm³; severe life-threatening Neutropenia

**Ethical Consideration**

This study was reviewed and approved by the Institutional Review Board (IRB) of Hawassa University College of Medicine and Health Science. A support letter was obtained from HUCSH Chief clinical director office and permission was obtained from the pediatrics ART clinic to collect the necessary data. Informed written consent was taken from the caretakers and assent was obtained from older children (above 8 years old) before enrollment in the study. Then the objective of this research was explained to the study participants, and those willing to participate were included. To ensure confidentiality of participant’s information, anonymous typing was applied. Each participant was interviewed alone to keep privacy. Test results were given to the clinicians who are working on the pediatric ART clinic of the hospital for further diagnosis and management.

**Results**

**Socio-Demographic and Clinical Characteristics of Study Participants**

A total of 273 HAART-experienced HIV-positive children were enrolled in this study. Of whom: 139 (50.9%) were females and 82.1% were urban residents. The majority, (80.6%) of the participants were above seven years old. The mean age and standard deviation (SD) of the study participant were 10.2 ± 3.2 years. Clinically, 49.5% of the participants were in WHO clinical stage I. From the total study participants, 89.4% were on AZT/3TC/EFV, 83.9% did not take other drug and 11.7% had CD4 cell count of less than or equal to 350 cells/mm³ (Table 1).
Table 1
Sociodemographic and clinical characteristics of HIV-infected children attending Hawassa University comprehensive specialized hospital from July to December 2019, Hawassa, Ethiopia, \( (N = 273) \).

| VARIABLES               | CATEGORY          | N = 273(%) |
|-------------------------|-------------------|------------|
| Age in years            | ≤ 7               | 53(19.4)   |
|                         | > 7               | 220(80.6)  |
| Sex                     | Male              |            |
|                         | Female            |            |
| Residence               | Urban             | 49(17.9)   |
|                         | Rural             |            |
| Educational status      | Did not begin     | 38(13.9)   |
|                         | Primary           | 219(80.2)  |
|                         | Secondary         | 16(5.9)    |
| Family size             | ≤ 3               | 46(16.8)   |
|                         | 4–7               | 192(70.3)  |
|                         | > 7               | 35(12.8)   |
| WHO stage               | 1 and 2           | 269(98.5)  |
|                         | 3 and 4           | 4(1.5)     |
| Diarrheal Disease       | Yes               | 0          |
|                         | No                | 273(100)   |
| Intestinal parasites    | Yes               | 1(0.4)     |
|                         | No                | 272(99.6)  |
| Malaria                 | Yes               | 1(0.4)     |
|                         | No                | 272(99.6)  |
| Temperature C°          | ≤ 37              | 261(95.6)  |
|                         | > 37              | 12(4.4)    |
| Other medication        | No                | 229(83.9)  |
|                         | Cotrimoxazole     | 31(11.4)   |
|                         | Amoxicillin       | 5(1.8)     |
|                         | Augmentin         | 2(0.7)     |
|                         | Nutritional supplement | 6(2.2) |
| VARIABLES          | CATEGORY        | N = 273(%) |
|--------------------|-----------------|------------|
| ART Drugs          | AZT/3TC/EFV     | 244(89.4)  |
|                    | ABC/3TC/CALETR  | 20(7.3)    |
|                    | TDF/3TC/CALETRA | 9(3.3)     |
| CD4 cell cells/mm³ | ≤ 350           | 32(11.7)   |
|                    | > 350           | 241(88.3)  |
| Viral load         | ≤ 150           | 57(20.1)   |
|                    | > 150           | 216(79.1%) |

Baseline hematological and immunological characteristics

The baseline, mean hemoglobin level of the study participants was 12 mg/dl and 40.7% of the study participants were anemic. However; the median and Interquartile range of CD4 + T lymphocyte cells was 18.4 and 12.3–26.8 respectively. Most of the study participants had a CD4 + T lymphocyte cell percentage of < 15% (Table 2).

Table 2
Baseline Hematological and immunological characteristics HIV-infected children (N = 273)

| VARIABLE                                      | VALUE                |
|-----------------------------------------------|----------------------|
| Mean(SD) Hb, Mg/dl                           | 12(1.6)              |
| Anemia, N (%)                                |                      |
| Yes                                           | 111(40.7)            |
| No                                            | 162(59.3)            |
| CD4 + T lymphocyte percentage N (%)          |                      |
| Median CD4 + T lymphocyte percentage &(IQR)   | 18.4(12.3–26.8)      |
| > 25%                                         | 72(26.4)             |
| 15–25%                                        | 99(36.3)             |
| < 15%                                         | 102(37.4)            |

CD = Cluster of differentiation; IQR = Interquartile range; Hb = hemoglobin; SD = Standard Deviation

The immunological progression of the participants was indicated by the increasing trend of the CD4 + T cell median percentage from 18.4% at baseline to 29.2% during the study period and similarly, the mean hemoglobin level also increased from 12 mg/dl at baseline to 13.1 mg/dl during the study period (Fig-1).
Prevalence of hematological abnormalities and its distribution by CD4 + T cell and viral load

Out of the total number of study participants, 18.3% had Neutropenia, 14.3% had Leucopenia, 4% had Thrombocytopenia, 2.9% had Pancytopenia and 11.4% had Anemia (Table 3).

In the current study, the association of the distribution of hematological abnormalities and different CD4 + cell percentage was observed and all forms of hematological abnormalities were highly prevalent among study participants with CD4 cell percentage of < 15%. However, it was statistically significant with leucopenia (P = 0.02), Leucocytosis (P = 0.02), and lymphopenia (P = 0.001) (Table 3). While when we compare the hematological abnormalities and different stratified (ND, < 150 and > 150) viral loads, all forms of hematological abnormalities were highly prevalent with study participants who had greater than 150 viral copies / mm3 and it was statistically significant with anemia (P = 0.002), Lymphopenia (P = < 0.001), and pancytopenia (P = 0.001) (Table 3).

Table 3
Prevalence of hematological abnormalities and its correlation with CD4 and viral load

| variables | Total N (%) | CD4 percentage | Viral load |
|-----------|-------------|----------------|------------|
|           |             | > 25 | 15–25 | < 15 | P-value | ND | < 150 | ≥ 150 | P-Value |
| Anemia    | 31(11.4)    | 18(10.9) | 8(11.1) | 5(13.9) | 0.85 | 13(8.0) | 4(7.5) | 14(24.5) | 0.002 |
| Leucopenia| 39(14.3)    | 19(11.5) | 10(13.9) | 10(27.8) | 0.02 | 18(11.0) | 7(13.2) | 14(24.5) | 0.05  |
| Leucocytosis| 13(4.8)    | 6(3.6) | 7(9.7) | 0 | 0.02 | 6(3.7) | 5(9.4) | 2(3.5) | 0.05  |
| Neutropenia| 50(18.3)    | 30(18.2) | 13(18.1) | 7(19.4) | 0.9 | 29(17.8) | 8(15.4) | 13(22.8) | 0.7   |
| Lymphopenia| 27(9.9)     | 11(6.7) | 6(8.3) | 10(27.7) | 0.001 | 4(2.5) | 6(11.3) | 17(29.8) | < 0.001 |
| Thrombocytopenia| 11(4) | 5(3.0) | 2(2.8) | 4(11.1) | 0.07 | 5(3.1) | 1(1.9) | 5(8.8) | 0.1   |
| Pancytopenia| 8(2.9)     | 5(3.0) | 1(1.4) | 2(5.5) | 0.5 | 1(0.01) | 1(1.9) | 6(10.5) | 0.001 |
| AOHA      | 91(33.3)    |       |       |       |     |       |       |       |       |

AOHA = At least one hematological abnormalities; ND = Not detectable; CD = cluster of differentiation
Distribution of hematological abnormalities by age and gender

Higher prevalence of anemia and Leucocytosis among children of age group < 5 years of age in the current study was indicated as (25%) and (18.8%) respectively. Leucopenia was highly prevalent among 5–10 years old children and Neutropenia and lymphopenia were higher among children within the age group of 11–14 years. Similarly higher prevalence of Neutropenia (21.6%), lymphopenia (12.3%), thrombocytopenia (5.3%), and pancytopenia (3.5%) was found among children of older than the age group of (11–14) years old.

The prevalence of hematological abnormalities such as anemia (11.5%), neutropenia (18.7%), and pancytopenia (3.6%) had higher distribution among female children than males, but; no statistically significant association was observed (Table 4).

Table 4
Distribution of hematological abnormalities by age and gender at HUCSH (N = 273)

| Variables | Total | Gender | Age (in years) | P-value | Male | Female | P-Value |
|-----------|-------|--------|----------------|---------|------|--------|---------|
| Anemia    | 31(11.4) |        | 4(25) | 17(11.9) | 10(8.8) | 0.2 | 15(11.2) | 16(11.5) | 0.9 |
| Leucopenia| 39(14.3) |        | 1(6.3) | 22(15.4) | 16(14.0) | 0.01 | 23(17.2) | 16(11.5) | 0.2 |
| Leucocytosis| 13(4.8) |        | 3(18.8) | 6(4.2) | 4(3.5) | 0.01 | 8(6.0) | 5(3.6) | 0.2 |
| Neutropenia| 50(18.3) |        | 2(12.5) | 23(16.1) | 25(21.9) | 0.9 | 24(17.9) | 26(18.7) | 0.4 |
| Lymphopenia| 27(9.9) |        | 0 | 13(9.1) | 14(12.3) | 0.3 | 15(11.2) | 12(8.6) | 0.5 |
| Thrombocytopenia| 11(4) |        | 2 | 3(2.1) | 6(5.3) | 0.7 | 6(4.5) | 5(3.5) | 0.7 |
| Pancytopenia| 8(2.9) |        | 0 | 4(2.8) | 4(3.5) | 0.7 | 3(2.2) | 5(3.6) | 0.5 |
| At least one abnormality | 91(33.3) | | | | | | | | |
Based on WHO grading; Neutropenia grading was indicated in the current study as severe, moderate and mild as indicated in the figure below (Fig. 2)

**Predictors of CD4 T + cell change among study participants**

In linear univariate analysis, among the factors that predicted the CD4 T cell count change; only viral load (B. = -4.9; R2 = 0.050.09; p-value = < 0.001 was found to be a predictor of CD4 + T cell change among children receiving HAART in the current study.

| variable                | Change in CD4 T cell percentage |
|-------------------------|---------------------------------|
|                         | R2     | t   | P-value | B(CI)           |
| Sex                     | 0.01   | 1.7 | 0.08    | 2.7(-0.3-5.6)   |
| Age                     | 0      | -0.2| 0.8     | -0.05(-0.5-0.4) |
| Residence               | 0.03   | 2.5 | 0.07    | 4.9(1.2–8.8)    |
| HAART regimen           | 0.005  | -1.2| 0.2     | -2.0(-5.5-1.4)  |
| Other medication        | 0.004  | -1.1| 0.3     | -1.1(-3.1-0.9)  |
| WAZ                     | 0.016  | -2.1| 0.06    | -1.3(-2.4-0.07) |
| HAZ                     | 0.001  | -0.5| 0.6     | -0.2(-0.5-0.6)  |
| WHO stage               | 0.01   | -1.7| 0.09    | -2.0(-4.3-0.3)  |
| Anemia                  | 0      | 0.07| 0.9     | 0.2(-4.6-4.9)   |
| Viral load              | 0.09   | -5.5| <0.001  |                  |
| Family size             | 0.009  | -1.6| 0.1     |                  |
| Pancytopenia            | 0      | -0.33| 0.7    |                  |

The immunological and clinical characteristics of children under this study also showed failures in both immunological and clinical characteristics as shown in the figure below.

**Discussion**

The baseline prevalence of anemia before the initiation of HAART in this study was 111(40.7%). This result was comparable with the studies reported from northwest Ethiopia (42.8%) (21) Uganda 47.8% (22) and Brazil (37.5%) (23). But; our result was lower than the Indian study 65.5% (24). It was also higher than the study done in Addis Abeba, Ethiopia (18.9%) (25). The difference could be due to the difference
in the WHO clinical stage, presence of opportunistic infections, nutritional status, population, and geographic difference might some of the factors.

The prevalence of anemia after initiation of HAART was (11.4%) which was in line with the study conducted in Addis Abeba, Ethiopia(10.4%) (25) but; lower than the study done in northwest Ethiopia (18.9%) (26) and Nigeria (54.2%) (27). This variation might be attributed to the differences in ethnicity, study designs, and time of the study. Besides, variation in age of the study participants, HAART status and cutoff value in defining anemia, local prevalence of parasitic infections such as malaria or hookworm, as well as local nutritional patterns might contribute to the variation in magnitude of anemia.

The prevalence of leukocytosis in our study was (4.8%) which is relatively comparable with the study done in North West Ethiopia (4.1%) (16) but; lower than the study done in Kenya (6.2%) (28). In this study, the prevalence of Neutropenia was (18.3%) which was higher than the study conducted in northwest Ethiopia (15.8%) (26), Bahir Dar, Ethiopia (9.8%) (29), Addis Abeba, Ethiopia (5.7%) (25) and Kenya (16%). The possible difference might be the difference in the immunological status of the study participants and sample size. Neutropenia has been widely reported to be associated with some HAART drugs, particularly combination drugs that included zidovudine. Prophylactic co-trimoxazole, which is often used in these patients to prevent opportunistic infections, is thought to be the cause of neutropenia through an unknown mechanism (30). Any infiltrative process involving the bone marrow (infection, malignancy) may produce granulocytopenia. In clinical practice, however, drug toxicity is responsible for most of the granulocytopenia seen in patients with HIV infection. AZT therapy is probably the most common cause of neutropenia in patients with HIV infection (31). The prevalence of leukopenia was 14.3% which is relatively comparable with the study done in northwest Ethiopia (13.5%) (26) but higher than the study done in Bahir dar, Ethiopia (4.5%).

In our study, the prevalence of thrombocytopenia was 4% which was higher than the study reported from Northwest Ethiopia (1.8%) (26), and Lagos Nigeria (2.5%) (32). But; lower than the study from Jimma, Ethiopia (7.8%) (33), Addis Abeba, Ethiopia (5.7%) (25), Bahir Dar Ethiopia (6.3%) (29), Zimbabwe (7.2%) (34), Kenya (21%) (35), Kenya (6.5%) (28), Mumbai, India (10%) (36), West Bengal, India (11%) (37), Nepal (17.9%) (38), and Uttar Pradesh, India (29.78%) (39).

The difference in the prevalence could be due to the difference in sample size, geographical location, and the cut off value used. The mechanism of thrombocytopenia in HIV infection is mainly due to ineffective platelet production and increased platelet destruction by the spleen. Other possible causes of thrombocytopenia in HIV patients are immune-mediated destruction, TTP, impaired hematopoiesis, and toxic effects of medications and infections(31).

The prevalence of pancytopenia was 2.9% which is in contrast with the study done in northwest Ethiopia, which reported zero percent pancytopenia, Bahir Dar, Ethiopia (0.9%)(29), and Zimbabwe (4.1%) (34).

The immunological and clinical failure among HAART-experienced children in the current study was 4% and 5.5% respectively. In which both of these parameters were lower than a retrospective cohort study
conducted in the Oromia region, Ethiopia (6.69%), and (12.26%) respectively (40).

In our study, the median percentage of CD4 + T cells showed an increasing trend from 18.4% at baseline to 29.2% during the study. This was comparable with the study reported from West Africa, the Gambia, which showed an increasing trend from 13% at baseline to 27% (41). Viral load was found to be the only predictor (p = < 0.001) of CD4 + T cell percentage change which was supported by other different studies.

This study does not address the iron status of study participants (Iron diagnostic tests such as serum iron, ferritin and total iron-binding capacity (TIBC)), Hemoglobinopathies, inherited membrane disorders, chronic diseases related to HIV, other nutritional deficiencies and duration of ART drug on immunological and hematological parameters because of lack of resources. Therefore, longitudinal studies should be conducted to assess hematological and immunological abnormalities to address associated factors among HAART- experienced HIV-infected children.

**Conclusion**

The baseline, mean hemoglobin level of the study participants was 12 mg/dl and 40.7% of the study participants were anemic. However; the median and Interquartile range of CD4 + T lymphocyte cells was 18.4 and 12.3–26.8 respectively. Most of the study participants had a CD4 + T lymphocyte cell percentage of < 15% and all forms of hematological abnormalities were highly prevalent with study participants who had greater than 150 viral copies / mm3 in the current study. The high prevalence of these hematological, immunological, and viral load abnormalities in HAART- experienced children may be a reflection of a possibly high prevalence of anemia amongst children in several parts of Ethiopia. However, the direct effect of these abnormalities on HIV related morbidity and mortality requires that particular attention should be paid to HIV positive children. For these children, prompt diagnosis of anemia, identification of the underlying cause(s) of persistent anemia in HAART experienced patients, as well as the implementation of appropriate interventions, are essential.

**Abbreviations**

AIDS, Acquired Immune Deficiency Syndrome; ART, Antiretroviral Treatment; AZT, Zidovudine; CBC, Complete Blood Count; CD4+, Cluster differentiation; FACS, Fluorescence-Activated Cell Sorting; HAART, Highly Active Antiretroviral Treatment; Hgb, Hemoglobin; HIV, Human Immunodeficiency Virus; WHO, World Health Organization; HUCSH, Hawassa University Comprehensive Specialized Hospital.

**Declarations**

**Ethics approval and consent to participate**

The study was ethically cleared by the Institutional Review Board (IRB) of Hawassa University College of medicine and health science in February 2017 and approved the proposal with (Reference number: IRB -057-14- 02/2017 and Institutional Review Board chairperson Ayalew Astatkie (Ph.D.)). An original copy
of the approval, as well as the consent and assent form, is available upon request. A permission letter was obtained from Hawassa University hospital administrations.

The purpose and importance of the study were explained to each study participant, parents, and guardians. Finally, informed written consent and assent were obtained from each study participant, and any information obtained during the study was kept with utmost confidentiality, and those found infected with bacterial meningitis were treated in the hospital.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

The study was supported by a small grant obtained from Hawassa University to cover the purchasing of reagents and supply and personnel coast covered by this small grant. No other grant was secured for this work.

Authors’ contributions

MM: Concept, design, and acquisition of data, collected and analyzed the data, involved in laboratory work, and wrote the draft of the manuscript.

DA: Concept, design, acquisition of data, supervised, involved in proposal development, review the final draft of the manuscript.

TB: Concept, design, acquisition, involved in the review of the final draft of the manuscript, and data analysis.

Acknowledgments

The authors would like to thank Hawassa University College of Medicine and Health Science for supporting and allowing them to conduct this research. We also extend our thanks to all ART clinic staff who help us during data collection and laboratory work. Last but not least we also thank the study participants.
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Figures
Figure 1

Baseline and current CD4 T cell percentage and hemoglobin level among children at HUCSH, Southern Ethiopia, from July to December 2019
Figure 2

Neutropenia Grading among HAART-Experienced children at HUCSH ART clinic (n=273).
Figure 3

Clinical and Immunological characteristics of HAART-experienced children at HUCSH (n=273).

Supplementary Files

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