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ORIGINAL RESEARCH

Magnitude of cytopenias among HIV-infected children in Bahir Dar, northwest Ethiopia: a comparison of HAART-naïve and HAART-experienced children

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Background: AIDS, caused by HIV, is a multisystem disease that affects hematopoiesis. The aim of this study was to assess cytopenias among HIV-infected children who had a follow-up at Felege Hiwot Referral Hospital, Bahir Dar, northwest Ethiopia.

Methods: An institution-based cross-sectional study was conducted between April and May 2013. Systematic random sampling method was used to select the study participants. Descriptive statistics, independent *t*-test as well as chi-square and logistic regression were used for analysis. A p-value <0.05 was considered as statistically significant.

Results: A total of 224 children (112 highly active antiretroviral therapy [HAART]-naïve and 112 HAART-experienced) participated in the study. The magnitude of anemia, thrombocytopenia, neutropenia, leukopenia and pancytopenia among HAART-naïve HIV-infected children were 30.4%, 9.8%, 8%, 4.5% and 1.8%, respectively. The overall prevalence of anemia, neutropenia, thrombocytopenia, leukopenia and pancytopenia were 29.5%, 8.9%, 8%, 4.5% and 1.4%, respectively. Cluster of differentiation-4 percentage and mean corpuscular volume were significantly different between HAART-experienced and HAART-naïve children. Being of younger age and severely immunosuppressed were risk factors of anemia.

Conclusion: Anemia was the most common cytopenia, followed by neutropenia. Severe immunosuppression and younger age were significantly associated with anemia. Therefore, emphasis should be given for investigation and management of cytopenias in HIV-infected children, particularly for those who are immunosuppressed and of younger age.

Keywords: anemia, children, cytopenia, HAART, HIV, leukopenia, neutropenia, pancytopenia, thrombocytopenia

Background

AIDS is caused by HIV and is characterized by progressive damage to the body's immune system, which results in a number of OIs, immunological and hematological complications.[1,2] Immunological complication due to CD4+ T-lymphocyte depletion is a hallmark of HIV infection.[3] Hematological manifestations are among the most common clinicopathological manifestations of HIV infection, and they have been documented as the second most common cause of morbidity and mortality in HIV patients.[4–6] These complications are generally marked with cytopenias and dysplasias of all major blood cell lines, leading to anemia, leukopenia, thrombocytopenia and neoplasms.[7–9]

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Despite the attempts made to clearly understand the hematopoiesis impairment mechanism(s), it remained an intractable problem because of the paucity of studies using a suitable experimental animal model that closely replicates human hematopoiesis during an ongoing HIV infection in vivo.[10] It has been evidenced that HIV-associated cytopenias seem to be dependent on the level of viral replication, OIs, liver cirrhosis, malignancies and the effects of the HAART used.[11,12] Involvement of the hematopoietic system tends to be more severe in advanced stages of the disease.[7,13] The incidence and severity of cytopenias are generally correlated to the stage of the disease. In addition, cytopenias can adversely affect ART outcomes and result in higher mortality.[12,14–16] In HIV-infected children, cytopenias are the common problems.[3,17–21] The pathophysiology of HIV-related cytopenias in childhood is not well understood, which may be due to the complicated and dynamic changes associated with normal hematological development in early life.[18] The frequency and severity of cytopenias vary, while the disease progresses from the asymptomatic carrier state to advanced symptomatic stages. The frequencies of anemia, leukopenia and thrombocytopenia in asymptomatic HIV-infected children were 20%, 10% and 15%, respectively, while in HIV-infected children at the AIDS stage, their proportions were 70%, 65% and 40%, respectively.[22]

Even though the use of HAART reduces the rate of mortality, therapy-related potential adverse events are becoming the major concern in the era of HAART, particularly in resource-limited counties where undernutrition is common.[23–26] Both HIV/AIDS and undernutrition affect immune function; HIV/AIDS, together with lack of essential micronutrients, leads to severe immune dysfunction. Furthermore, compromised immune status increases susceptibility to infectious diseases and profoundly complicates cytopenias and their management.[27,28] A number of studies have been conducted on cytopenias among adult HIV-infected patients before and after the initiation of HAART. However, in HIV-infected children, limited data are available, and these are not much well elucidated, especially in developing countries. Moreover, there are controversial reports regarding the efficacy and impact of HAART in resolving immunological and hematological complication in HIV patients.[29–31] Thus, this study was aimed to assess the cytopenias among HAART-naïve and HAART-experienced HIV-infected children.

Patients and methods

Study setting, population, sample size and sampling procedure

A cross-sectional study was conducted at Bahir Dar Felege Hiwot Referral Hospital, northwest Ethiopia, between April and May 2013. Felege Hiwot Referral Hospital is found in Bahir Dar, which is located 565 km away from Addis Ababa. Geographically, the city is located between 9°20′ and 14°20′ north latitudes and between 30°20′ and 40°20′ east longitudes and is at an altitude of 1,830 m above sea level. The hospital serves >5 million people and provides comprehensive health care services, including ART treatment and monitoring for both pediatric and adult people living with HIV/AIDS.

The study population comprised HIV-infected children who had been followed up at the Pediatric ART Clinic of Felege Hiwot Referral Hospital during the study period. HIV-infected children who were HAART-naïve and HAART-experienced for at least 6 months were eligible to be included in the study. Children who had been previously confirmed as having chronic renal failure and liver disease prior to HIV infection, as well as those who underwent radiation therapy and/or immunosuppressive chemotherapy in the previous 45 days, were excluded from the study due to the fact that these may unambiguously affect the hematological values.

For sample size determination, double population proportional formula was used by considering the following assumptions: 2-sided confidence level at 95%, power of 80% and 1:1 ratio of HAART-experienced:HAART-naïve children. We used a 21.9% prevalence rate of anemia for HAART-experienced children, as per a study conducted in Jimma, Ethiopia,[32] and 40% for HAART-naïve children (a default value of OpenEpi) to get the maximum sample size. Then, a total of 224 HIV-infected children (112 HAART-naïve and 112 HAART-experienced for at least 6 months) were included in the study.

A systematic random sampling technique was used. On a daily basis, an average of 9 HAART-naïve and 12 HAART-experienced children were getting health care service in the Pediatric ART Clinic of Felege Hiwot Referral Hospital. A total of 840 HIV-infected children (360 HAART-naïve and 480 HAART-experienced) visited the ART clinic during the study period. Every third HAART-naïve HIV-infected child from the sequence of ART visitors was included in the study. Similarly, every fourth HAART-experienced HIV-infected child was included.

Data collection and laboratory analysis

Sociodemographic and socioeconomic characteristic of children and their caregivers were collected using a structured questionnaire via a face-to-face interview technique. Clinical data were collected by reviewing the medical records of HIV-infected children. The aforementioned data were collected by trained clinical nurses working in the Pediatric ART Clinic. Weight and height were measured, and the weight-for-age

status and height-for-age status were scored from the child's growth monitoring chart.

Venous blood (4 mL) was collected from each study participant using test tubes containing ethylenediaminetetraacetic acid following aseptic procedures. Part of the blood sample was analyzed using Cell DYN 1800 (Abbott Laboratories, Abbott Park, IL, USA) for the determination of hematological parameters, which include RBC parameters (RBC count, Hg level, MCH, MCHC, MCV and RDW%); WBC parameters (total WBC count, ANC, neutrophil percentage, lymphocyte count, lymphocyte percentage, mid count that encompasses eosinophil, basophil and monocyte and mid cell percentage) and platelet parameter (platelet count and MPV). The remaining blood sample was analyzed for the determination of CD4+ T-cell value using fluorescence-activated cell sorter counter (BD, San Jose, CA, USA). While doing all laboratory analyses, the standard operating procedure, daily maintenance, weekly maintenance and internal quality control procedure were strictly followed throughout the research process.

Assessment of cytopenias and immunological status

HIV-associated immunodeficiency was defined using the World Health Organization (WHO) age-related CD4 value stratification for HIV-infected infants and children.[33] Mild immunodeficiency was defined as CD4% of 30% to <35% for infants <11 months, CD4% of 25% to <30% for children of age 12–35 months, CD4% of 20% to <25% for children aged 36–59 months and CD4 count of 350–499 cells/mm^3 for children aged >5 years. Advanced immunodeficiency was defined as follows: CD4% of 25% to <30% for infants <11 months, CD4% of 20% to <25% for children aged 12–35 months, CD4% of 15% to <20% for children aged 36–59 months and CD4 count of 200–349 cells/mm^3 for children aged >5 years. Severe immunodeficiency was also defined as follows: CD4% <25% for infants <11 months, 15% to <20% for children aged 12−35 months and <15% for children aged >3 years.[33]

Anemia was defined based on the WHO criteria after Hg has been adjusted for altitude and was stratified based on age (Hg <11.0 g/dL for children aged 6–59 months, Hg <11.5 g/dL for children aged 5–11 years and Hg <12.0 g/dL for children aged ≥12 years). Mild anemia was defined as follows: Hg 10.0–10.9 g/dL for children aged 5–59 months, 11.0–11.4 g/dL for children aged 5–11 years and 11.0–11.9 g/dL for children aged 12–14 years. Moderate anemia was defined as Hg 7.0–9.9 g/dL for children aged 5–59 months and 8–10.9 g/dL for children aged 5–14 years. Severe anemia was also defined as Hg <7.0 g/dL for children aged 6–59 months and <8.0 g/dL for those aged 5–14 years.[34]

Leukopenia was defined as a total WBC count <3,000 cells/mm^3.[9] Thrombocytopenia and thrombocytosis were defined as a platelet count <150,000/mm^3 and platelet count >450 × 10^3 cells/mm^3, respectively.[32] Neutropenia was also defined as absolute neutrophil count of <1,000/mm^3, and the severity has also been classified as mild, moderate and severe.[32]

Statistical analysis

Data were cleaned, sorted, categorized, coded and entered into Epi Info version 3.5.1. The data were transferred to SPSS version 20 for analysis. Descriptive statistics were obtained and the results are presented in Tables 1–6 and Figure 1. Normality of data was checked; and chi-square and independent *t*-tests were used to compare the mean hematological values between the HAART-naïve and HAART-experienced HIV-infected children. Bivariate logistic regression analyses were carried out for the cytopenias, and variables having *p*-value <0.2 in bivariate logistic analysis were included in the multivariable logistic analysis model to assess the association between cytopenias and explanatory variables. Odds ratios (ORs) with 95% CIs were used to measure the strength of the statistical associations. A *p*-value <0.05 was considered statistically significant.

Ethical considerations

This study was approved by the College of Medicine and Health Sciences Research Ethical Committee and the Institutional Review Board of the University of Gondar. The purpose and importance of the study was explained to each caregiver. Informed written consent was taken from the caregivers, and in addition, assent was obtained from children aged >7 years before the commencement of the study. To ensure confidentiality of participants and their information, anonymous typing was used whereby the name of the participants and any participants' identifiers were not written on the questionnaire. The participants were interviewed alone to maintain their privacy. Laboratory findings of study participants were communicated with the responsible clinicians assigned at the Pediatric ART Clinic.

Results

Sociodemographic characteristics

A total of 224 study participants were enrolled in this study. The median age of the study participant was 8 years (interquartile range: 6 years). More than half of the study participants, 126 (56.3%), were males. Among the study participants, 180 (80.4%) were from urban setting and 96 (42.9%) were attending primary school. A majority, 157 (70.1%), of caregivers earned monthly income <1,400 ETB (Table 1).

Table 1 Sociodemographic characteristics of caregivers/guardians and HIV-infected children at the Pediatric ART Clinic, Felege Hiwot Referral Hospital, Bahir Dar, northwest Ethiopia, 2013

Characteristics	HAART-naïve	HAART-experienced	Total
	n (%)	n (%)	N (%)
Age of child, years			
≤5	32 (28.6)	24 (21.4)	56 (25)
6–10	53 (47.3)	53 (47.3)	106 (47.3)
11–14	27 (24.1)	35 (31.2)	62 (27.7)
Sex of child			
Male	67 (59.8)	59 (52.7)	126 (56.3)
Female	45 (40.2)	53 (47.3)	98 (43.7)
Residence of child			
Urban	89 (79.5)	91 (81.25)	180 (80.4)
Rural	23 (20.5)	21 (18.75)	44 (19.6)
Educational status of children			
Nursery	15 (13.4)	9 (8)	24 (10.7)
Kindergarten	32 (28.6)	26 (23.2)	58 (25.9)
Primary school (1–4)	51 (45.5)	45 (40.2)	96 (42.9)
Elementary (5–8)	14 (12.5)	32 (28.6)	46 (20.5)
Whom the child lives with			
Parents	72 (64.3)	71 (63.4)	143 (63.8)
Father only	7 (6.2)	15 (13.4)	22 (9.8)
Mother only	23 (20.5)	18 (16)	41 (18.3)
Grandparents	2 (1.8)	2 (1.8)	4 (1.8)
Guardian	1 (0.9)	1 (0.9)	2 (0.9)
Relative	5 (4.5)	3 (2.7)	8 (3.6)
Caregiver	2 (1.8)	2 (1.8)	4 (1.8)
Parental status			
Both alive	73 (65.2)	77 (68.7)	150 (67)
Father dead	18 (16.1)	10 (8.9)	28 (12.5)
Mother dead	6 (5.3)	16 (14.3)	22 (9.8)
Separated	8 (7.1)	6 (5.4)	14 (6.2)
Both mother and father dead	7 (6.3)	3 (2.7)	10 (4.5)
Family size, n			
≤3	52 (46.4))	46 (41.1)	98 (43.8)
4–5	54 (48.2)	54 (48.2)	108 (48.2)
>5	6 (5.4)	12 (10.7)	18 (8)
Monthly family income, ETB			
Lowest	75 (67)	82 (73.2)	157 (70.1)
Middle	36 (32.1)	27 (24.1)	63 (28.1)
High	1 (0.9)	3 (2.7)	4 (1.8)
Caregiver's educational status			
Unable to read and write	19 (17)	42 (37.5)	61 (27.2)
Able to read and write only	22 (19.6)	14 (12.5)	36 (16.1)
Primary school	18 (16.1)	16 (14.3)	34 (15.2)
High school	23 (20.5)	17 (15.2)	40 (17.9)
Tertiary education	30 (26.8)	23 (20.5)	53 (23.7)
Caregiver occupational status			
Privately employed	43 (38.4)	44 (39.3)	87 (38.8)
Government employed	23 (20.5)	35 (31.2)	58 (25.9)
Merchant	26 (23.2)	16 (14.3)	42 (18.8)
Farmer	20 (17.9)	17 (15.2)	37 (16.5)

Abbreviations: ART, antiretroviral treatment; ETB, Ethiopian Birr; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus.

Medical characteristics of the study participants

Among the participants, 64 (57.2%) of HAART-naïve and 47 (41.9%) of HAART-experienced children were in WHO clinical stage I. Thirty (26.8%) and 39 (34.8%) of HAART-naïve children were underweight and stunted, respectively, whereas 44 (39.3%) and 31 (27.7%) HAART-experienced children were underweight and stunted, respectively. Moreover, 19 (17%), 16 (14.3%), 15 (13.4%), 15 (13.4%), 14 (12.5%) and 9 (8%) of HAART-naïve children presented with fever, skin rash, diarrhea, OIs, pneumonia and oral thrush, respectively. Likewise, 14 (12.5%), 13 (11.6%), 10 (9%), 10 (9%), 9 (8%) and 8 (7.1%) of HAART-experienced children presented with skin rash, fever, pneumonia, OIs, gastroenteritis and diarrhea, respectively. Severe, advanced and moderate immunosuppression was observed among 17.9%, 12.9% and 32.1% of the study participants, respectively (Table 2).

Cytopenias

Of the total study participants, 66 (29.5%), 20 (8.9%), 18 (8%), 10 (4.5%) and 3 (1.4%) were anemic, neutropenic, thrombocytopenic, leukopenic and pancytopenic, respectively. The occurrence of anemia, thrombocytopenia, neutropenia, leukopenia and pancytopenia among HAART-naïve HIV-infected children was reported to be 30.4% (n=34), 9.8% (n=11), 8% (n=9), 4.5% (n=5) and 1.8% (n=2), respectively (Figure 1).

Comparison of hematological profile

The mean values (±SD) of RBC, Hg, WBC, ANC, lymphocyte count, platelet count and absolute CD4 count in HAART-naïve children were 4.48±0.58 × 10^6/μL, 13.25±3.03 g/dL, 8.18±3.32 × 10^3/μL, 3.94±2.26 × 10^3/μL, 3.46±2.2 × 10^3/μL, 297.91±107.67 × 10^3/μL and 873.16±446.57/μL respectively. In children who were HAART-experienced, the mean values (±SD) were 4.94±4.75 × 10^6/μL, 13.21±1.6 g/dL, 8.01±3.26 × 10^3/μL, 3.84±2.16 × 10^3/μL, 3.75±5.17 × 10^3/μL, 300.99±106.16 × 10^3/μL and 767.86±486.6/μL, respectively.

On the basis of the mean values, the data indicated that Hg, RBC count, WBC count and platelet count did not show statistically significant differences between HAART-naïve and HAART-experienced HIV-infected children. However, there was a statistically significant difference in CD4% and MCV values between HAART-naïve and HAART-experienced children ($p<0.05$) (Table 3).

Table 2 Medical characteristics of HIV-infected children at the Pediatric ART Clinic, Felege Hiwot Referral Hospital, Bahir Dar, northwest Ethiopia, 2013 (N=224)

Clinical characteristic	HAART-naïve	HAART-experienced	Total
	n (%)	n (%)	N (%)
WHO staging			
I	64 (57.2)	47 (41.9)	111 (49.6)
II	36 (32.1)	32 (28.6)	68 (30.3)
III	12 (10.7)	30 (26.8)	42 (18.8)
IV	0	3 (2.7)	3 (1.3)
Immune status			
Severe immunosuppression	17 (15.2)	23 (20.5)	40 (17.9)
Advanced immunosuppression	12 (10.7)	17 (15.2)	29 (12.9)
Mild immunosuppression	40 (35.7)	32 (28.6)	72 (32.1)
Normal immune status	43 (38.4)	40 (37.7)	83 (37.1)
Weight-for-age status			
Underweight	30 (26.8)	44 (39.3)	74 (33)
Normal	82 (73.2)	68 (60.7.)	150 (67)
Height-for-age status			
Stunted	39 (34.8)	31 (27.7)	70 (31.3)
Normal	73 (65.2)	81 (72.3)	154 (68.7)
OIs			
Yes	15 (13.4)	10 (9)	25 (11.2)
No	97 (86.6)	102 (91)	212 (88.8)
Gastroenteritis			
Yes	6 (5.4)	9 (8)	15 (6.7)
No	106 (94.6)	103 (92)	209 (93.3)
Pneumonia			
Yes	14 (12.5)	10 (9)	24 (10.7)
No	98 (87.5)	102 (91)	200 (89.3)
Oral thrush			
Yes	9 (8)	5 (4.5)	14 (6.2)
No	103 (92)	107 (95.5)	210 (93.8)
Skin rash			
Yes	16 (14.3)	14 (12.5)	30 (13.4)
No	96 (85.7)	98 (87.5)	194 (86.4)
Fever			
Yes	19 (17)	13 (11.6)	32 (14.3)
No	93 (83)	99 (88.4)	192 (85.7)
Presence of diarrhea			
Yes	15 (13.4)	8 (7.1)	23 (10.3)
No	97 (86.6)	104 (92.9)	201 (89.7)
Kind of diarrhea			
Acute	8 (53.3)	7 (87.5)	15 (65.2)
Chronic	7 (46.7)	1 (12.5)	8 (34. 8)

Abbreviations: ART, antiretroviral treatment; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; OIs, opportunistic infections; WHO, World Health Organization.

Immune status and cytopenias

In this study, the prevalence of anemia among cases with severe, advanced and mild immunosuppression was 19 (47.5%), 5 (17.2%) and 19 (26.4%), respectively. Furthermore, leukopenia and thrombocytopenia were found in 7 (17.5%) and 4 (10%) of cases with severe immune suppression (Table 4).

Comparison of cytopenias

The overall prevalence of anemia among HIV-infected children was 66 (29.5%). Anemia was found in 34 (30.5%) and 32 (28.6%) of HAART-naïve and HAART-experienced children, respectively. Among anemic cases, 1 (2.9%) of HAART-naïve and 2 (6.3%) of HAART-experienced children had severe anemia. About 11 (9.8%), 9 (8%) and 5 (4.5%) of HAART-naïve HIV-infected children were thrombocytopenic, neutropenic and leukopenic, respectively. Moreover, 11 (9.8%), 7 (6.2%) and 5 (4.5%) of HAART-experienced HIV-infected children were neutropenic, thrombocytopenic and leukopenic, respectively (Table 5).

Anemia and associated factors

In bivariate analysis, age, severe immunosuppression and presence of bleeding were significantly associated with

Table 3 Comparison of hematological profiles between HAART-naïve and HAART-experienced HIV-infected children at Pediatric ART Clinic of Felege Hiwot Referral Hospital in Bahir Dar, northwest Ethiopia, 2013 (N=224)

Hematological values				
Variables	**HAART-naïve**	**HAART-experienced**	**Total**	***p*-value**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	
Hg, g/dL	13.25±3.03	13.21±1.60	13.23±2.41	0.936
RBC, $\times 10^6$/μL	4.48±0.58	4.94±4.75	4.71±3.39	0.319
HCT, %	38.21±4.85	38.43±4.00	38.32±4.44	0.708
MCV, fL	86.67±13.17	90.10±11.6	87.88±12.59	0.008**
MCH, pg	29.88±5.46	31.11±4.07	30.50±4.85	0.057
MCHC, g/dL	33.92±1.96	34.09±3.74	34.01±2.9	0.671
RDW, %	15.11±2.57	14.76±2.18	14.94±2.39	0.264
WBC, $\times 10^3$/μL	8.18±3.32	8.01±3.26	8.09±3.29	0.708
ANC, $\times 10^3$/μL	3.94±2.26	3.74±2.05	3.84±2.16	0.499
Lymphocyte count, $\times 10^3$/μL	3.46±2.2	3.74±5.17	3.6±3.97	0.603
Mid (absolute), $\times 10^3$/μL	1.34±1.7	2.21±8.10	1.76±5.77	0.247
Neutrophil, %	45.22±11.98	45.24±14	45.23±13.01	0.992
Lymphocyte, %	41.31±11.67	41.67±12.67	41.49±12.21	0.827
Mid, %	13.41±5.17	12.51±4.81	12.96±5	0.178
PLT, $\times 10^3$cells/μL	297.91±107.67	300.99±105.10	299.45±06.16	0.829
MPV, %	9.72±1.92	9.82±7.96	9.77±5.77	0.899
CD4, cells/μL	873.16±466.53	767.86±486.60	820.51±78.52	0.100
CD4, %	28.73±14.22	24.78±10.69	26.73±12.71	0.018*

Note: *Significant at $p<0.05$; **significant at $p<0.01$ by independent *t*-test analysis.
Abbreviations: ANC, absolute neutrophil count; ART, antiretroviral treatment; CD4, cluster of differentiation-4; HAART, highly active antiretroviral therapy; HCT, hematocrit; HIV, human immunodeficiency virus; Hg, hemoglobin; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; mid, mixed cell; MPV, mean platelet volume; PLT, platelets; RBC, red blood cell; RDW, red cell distribution width; SD, standard deviation; WBC, white blood cell.

Table 4 Cytopenias with regard to immune status of HIV-infected children at Pediatric ART Clinic, Felege Hiwot Referral Hospital, Bahir Dar, northwest Ethiopia, 2013 (N=224)

Cytopenic status	Immunosuppression			
	No (%)	**Mild (%)**	**Advanced (%)**	**Severe (%)**
Anemic status				
Anemic	23 (27.7)	19 (26.4)	5 (17.2)	19 (47.5)
Nonanemic	60 (72.3)	53 (73.6)	24 (82.8)	21 (42.5)
Leukopenic status				
Leukopenic	7 (8.4)	3 (4.2)	0	0
Nonleukopenic	76 (91.6)	69 (95.8)	29 (100)	40 (100)
Neutropenic status				
Neutropenic	10 (12)	9 (12.5)	0	1 (2.5)
Nonneutropenic	73 (88)	63 (87.5)	29 (100)	39 (97.5)
Thrombocytopenic status				
Thrombocytopenic	7 (8.4)	6 (8.3)	1 (3.4)	4 (10)
Nonthrombocytopenic	76 (91.6)	66 (91.7)	28 (96.6)	36 (90)

Abbreviations: ART, antiretroviral treatment; HIV, human immunodeficiency virus.

anemia. But in multivariate logistic regression analysis, controlling the possible cofounders, age of ≤5 years (adjusted odds ratio [AOR] =4.3, 95% CI: 1.7–10.9), age of 6–10 years (AOR =3.1, 95% CI: 1.3–7.2) and severe immunosuppression (AOR =2.95, 95%CI: 1.26–6.9) remained risk factors of anemia in HIV-infected children (Table 6).

Discussion

Cytopenia is the most common manifestation of advanced HIV infection.[13,17,34] It is proposed that they are caused by the impaired growth and development of hematopoietic progenitor cells in the bone marrow due to the presence of HIV proteins and changes in the cytokine expression, which potentially lead to an altered maturation process and increased cell death of ≥1 bone marrow cell lineages.[13,35] These abnormalities have been directly correlated with the degree of immunosuppression and disease progression.[15,36–38] It has also been documented that they potentially limit the efficacy of HAART treatment and strongly predict morbidity and mortality in HIV-infected individuals.[39–43]

In this study, the prevalence of anemia was found to be 29.5%, making it more common than neutropenia, thrombocytopenia, leukopenia and cytopenia. This is in agreement with most of the literatures.[9,32,44] Experimental studies suggest that dyserythropoiesis and early apoptosis of red cell precursors are common features in HIV infection.[45] In addition, HIV infection causes deficiency and defects in metabolism of iron, vitamin B12 and other micronutrients, which may lead to anemia.[46,47] Moreover, immune suppression associated with HIV infection can be a cause for the onset of chronic inflammation and/or chronic disease, such

Table 5 Cytopenias and other hematologic abnormalities in HIV-infected children with respect to HAART status at Pediatric ART Clinic, Felege Hiwot Referral Hospital, Bahir Dar, northwest Ethiopia, 2013 (N=224)

Variable	HAART status		Total (%)	*p*-value
	HAART-naïve (%)	HAART-experienced (%)		
Anemic status				
Anemic	34 (30.4)	32 (28.6)	66 (29.5)	0.769
Nonanemic	78 (69.6)	80 (71.4)	158 (70.5)	–
Severity of anemia				
Mild	16 (47.1)	21 (65.6)	37 (56.1)	0.315[#]
Moderate	17 (50)	9 (28.1)	26 (39.4)	–
Severe	1 (2.9)	2 (6.3)	3 (4.5)	–
RBC size				
Microcytic RBC	38 (33.9)	18 (16.1)	56 (25)	0.016
Normocytic RBC	61 (54.5)	76 (67.8)	137 (61.2)	–
Macrocytic RBC	13 (11.6)	18 (16.1)	31 (13.8)	–
RBC Hg content				
Hypochromic RBC	28 (25)	12 (10.7)	40 (17.1)	–
Normochromic RBC	75 (70)	86 (75.8)	161 (71.9)	0.019
Hyperchromic RBC	9 (5)	14 (12.5)	23 (10.3)	–
Leukopenic status				
Leukopenic	5 (4.5)	5 (4.5)	10 (4.5)	0.63
Nonleukopenic	107 (95.5)	107 (95.5)	214 (95.5)	–
Neutropenic status				
Neutropenic	9 (8)	11 (9.8)	20 (8.9)	0.41
Nonneutropenic	103 (92)	101 (90.2)	104 (91.1)	–
Severity of neutropenia				
Mild	8 (88.9)	9 (81.8)	17 (85)	0.579[#]
Moderate	1 (11.1)	2 (18.2)	3 (15)	–
Thrombocytopenic status				
Thrombocytosis	8 (7.2)	6 (5.3)	14 (6.3)	0.506
Normal	93 (83.0)	99 (88.4)	192 (85.7)	–
Thrombocytopenic	11 (9.8)	7 (6.3)	18 (8)	–

Note: [#]Analysis done by Fisher's exact test.
Abbreviations: ART, antiretroviral treatment; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; Hg, hemoglobin; RBC, red blood cell.

Table 6 Factors associated with anemia among HAART-naïve and HAART-experienced children at Pediatric ART Clinic, Felege Hiwot Referral Hospital, northwest Ethiopia, April–May 2013 (N=224)

Variable	Anemic status		Total (%)	COR (95% CI)	*p*-value	AOR (95% CI)	*p*-value
	Anemic (%)	Nonanemic (%)					
Age, years							
<5	24 (42.9)	32 (57.1)	56 (25)	4.42 (1.83–10.68)	0.001	4.3 (1.7–10.9)	0.002
6–10	33 (31.1)	73 (68.9)	106 (47.3)	2.66 (1.18–6.03)	0.019	3.1 (1.3–7.2)	0.011
11–14	9 (14.5)	53 (85.5)	62 (27.7)	1	–	1	–
Gastroenteritis							
Yes	7 (53.8)	6 (46.2)	13 (5.9)	3.01 (0.97–9.32)	0.057	2.9 (0.3–1.05)	0.092
No	59 (28)	152 (72)	152 (94.1)	1	–	1	–
Bleeding							
Yes	3 (11.5)	23 (88.5)	26 (11.6)	0.28 (0.08–0.97)	0.044	0.3 (0.08–1.05)	0.059
No	63 (31.8)	135 (68.2)	198 (88.4)	1	–	1	–
Immunological status							
Severe immunosuppression	19 (47.5)	21 (52.5)	40 (17.9)	2.36 (1.08–5.2)	0.032	2.95 (1.26–6.9)	0.041
Advanced immunosuppression	5 (17.5)	24 (82.8)	29 (12.9)	0.54 (0.0.2–1.6)	0.27	0.67 (0.22–2.06)	0.48
Mild immunosuppression	19 (26.4)	53 (73.6)	72 (32.1)	0.94 (0.5–1.9)	0.85	1.25 (0.59–2.66)	0.55
No immunosuppression	23 (27.7)	60 (72.3)	83 (37.1)	1	–	1	–

Abbreviations: AOR, adjusted odds ratio; CI, confidence interval; COR, crude odds ratio; HAART, highly active antiretroviral therapy.

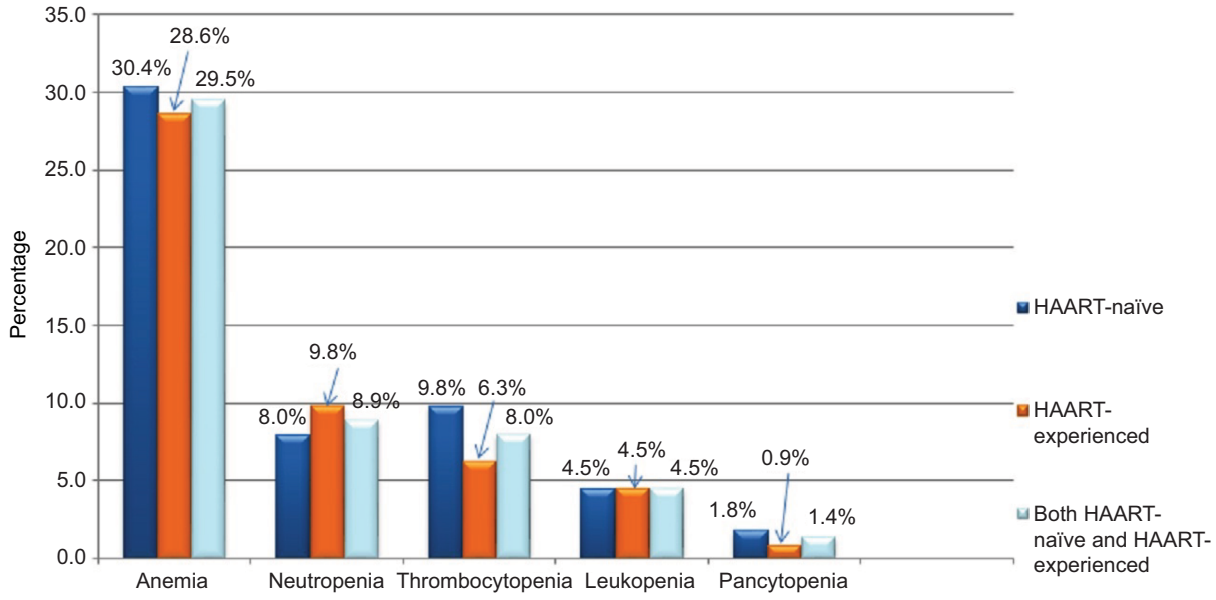

Figure 1 Frequency of cytopenias in HIV-infected children at Pediatric ART Clinic, Felege Hiwot Referral Hospital, Bahir Dar, northwest Ethiopia.
Abbreviations: ART, antiretroviral treatment; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus.

as tuberculosis, recurrent diarrheal diseases, recurrent bacterial pneumonia and viral infections, which can result in anemia as well as other cytopenias. The prevalence of anemia (29.5%) is lower than that reported in Lagos (77.9%),[9] Nepal (74.4%),[48] West Bengal, India (69%),[37] Uganda (57.6%)[49] and Dar es Salaam, Tanzania (44%),[18] and it is higher than that in Addis Ababa, Ethiopia (22.2%),[50] Jimma, Ethiopia (21.9%),[32] Gondar, Ethiopia (16.2%)[51] and Enugu, Nigeria (3%).[52] This variation might be attributed to the differences in ethnicity, study designs and time of study. In addition, variation in age of the study participants, HAART status and cutoff value in defining anemia, local prevalence of parasitic infections such as malaria or hookworms, as well as local nutritional patterns might contribute to the variation in magnitude of anemia.

In this study, the number of cases with mild, moderate and severe anemia was 37 (56.1%), 26 (39.4%) and 3 (4.5%), respectively. This is comparable with the results of another study done in Gondar, Ethiopia,[51] where occurrence of mild, moderate and severe anemia was 60.5%, 37.2% and 2.3%, respectively. Similarly, it is in agreement with a study done in Addis Ababa, Ethiopia,[50] where mild, moderate and severe anemia were reported to be 52.2%, 42.5% and 5%, respectively. The proportion of severe anemia in our studies is lower than that in studies conducted in Jimma, Ethiopia,[32] and Dar es Salaam, Tanzania,[18] which were reported to be 14.3% and 15%, respectively. The low magnitude of severe anemia in the current study might be related to the improved level of societal awareness about the positive implication of HIV monitoring and treatment, improved access to HIV monitoring and treatment facilities as well as updating of HIV monitoring and therapeutic modalities over time.

In the current study, the second most common cytopenia was neutropenia, which was observed in 8.9% HIV-infected children. The possible explanation may be the fact that HIV directly infects bone marrow and bone marrow stromal cell, which may reduce hematopoiesis. In addition, it is speculated that the decline in vitamin B12 and the presence of antibodies to HIV envelope glycoprotein 120 suppress bone marrow progenitor cells, in addition to being implicated as a causal factor of cytopenia, including neutropenia.[20] Furthermore, ART used to suppress the viral load may adversely affect the hematopoietic capacity of bone marrow.[12] Compared to studies done in Lagos, Nigeria, (17.5%)[9] and West Bengal, India (19%),[37] the prevalence of neutropenia is lower. The possible difference might be the difference in immunological status of the study participants and sample size. In the study from Lagos, Nigeria, of the total 68 children who participated in the study, about 75% were in the Centers for Disease Control and Prevention, USA (CDC) clinical stages B and C. Likewise, in the West Bengal study, of the total 100 children participating in the study, 50% were in WHO stages 3 and 4. But, in the current study, 20% of the study participants were in WHO stages 3 and 4.

In this study, thrombocytopenia was found to be 8%, which is the third most common cytopenia in HIV-infected children. The possible biological explanations for why thrombocytopenia is common in HIV infection might be due to an increased platelet destruction either caused by the nonspecific deposition of circulating immune complexes on platelets or the presence

of specific antiplatelet glycoprotein antibodies, leading to immune-mediated thrombocytopenia, as well as direct HIV infection of megakaryocytes and their precursors, resulting in higher thrombocytopenia[15,53] The result is comparable to studies done in Mumbai, India (10%)[54] and West Bengal, India (11%).[37] However, it is higher than that in the report from Lagos (2.5%);[9] and lower than studies done in Nepal (17.9%),[48] Kenya (21%)[44] and Uttar Pradesh, India (29.78%).[55] The variations in prevalence could be attributed to the difference in the reference range used to define thrombocytopenia as Adetifa et al[9] used platelet count $<100 \times 10^3/mm^3$; HAART status, as Kibaru et al[44] included children who were HAART-naïve; and sample size, as Poudel et al[48] and Kumar et al[55] used small sample size to determine the magnitude of thrombocytopenia.

In the current study, 4.5% of study participants were leukopenic. This is comparable with a study done in Lagos, Nigeria (6%).[9] However, it is lower than that in studies done at Kenyatta hospital, Kenya (10%)[44] and West Bengal, India (34%),[37] and higher than that in the study done in Mumbai, India (2%).[54] The possible reason for the variation in magnitude of leukopenia might be due to the differences in ethnicity, age of study participants included in study, HAART status as well as prevalence of infectious and noninfectious diseases.

In this study, severe immunosuppression (AOR =2.95, 95% CI: 1.26–6.9) was significantly associated with anemia. Similar findings had been reported previously in Addis Ababa, Ethiopia,[50] Western Uganda,[49] and South India,[46] revealing that there was statistically significant association between immune suppression and anemia. This could be explained by the fact that immune suppression potentially leads to viral replication, which may cause anemia through increased cytokine-mediated myelosuppression and higher burden of OIs.[13,56] Furthermore, age <5 years (AOR =4.3, 95% CI: 1.7–10.9) and age of 6–10 years (AOR =3.1, 95% CI: 1.3–7.2) also remained risk factors of anemia. This is also similar with other studies.[18,49,57] The possible reason for increased risk of anemia in younger children may be related with the high nutritional requirement for production of RBCs and the high frequency of comorbidity because their immune status is not well developed. Unlike anemia, neutropenia, thrombocytopenia and leukopenia were not significantly associated with independent variables. The possible explanation could be the small number of cases of children who had these cytopenias and the small sample size; therefore, the number of observations in each category of independent variables would be small and these observations would have low power to predict association.

In the current study, the mean MCV value in HAART-experienced HIV-infected children was significantly higher than in children who were HAART naïve. The possible reason for the high MCV value in HAART-experienced HIV-infected children might be related with the use of zidovudine-based first-line HAART. In this study, of the total 18 children who were on first-line HAART having MCV value >100 fL, 11 (61%) were taking zidovudine-based ART regimen. Evidence suggests that zidovudine causes marrow erythroid hypoplasia, aplasia and megaloblastic maturation, which can be accompanied by a progressive raise in erythrocyte MCV.[58,59] The other possible reason for the difference would be related to the nutritional deficiencies, particularly iron deficiency, the most common nutritional deficiency in developing countries,[60] which causes microcytic–hypochromic RBC morphology in HAART-naïve children.

The MCV could show an increment due to iron supplements and nutritional modification provided after HAART initiation. Together with the macrocytosis that appears to be overt after they are initiated into zidovudine-containing regimens, the MCV value shows significant elevation. Our data also supported that microcytosis is significantly higher in HAART-naïve (33.9%), compared to HAART-experienced, HIV-infected children (16.1%). A retrospective study done in Kenya has shown that, compared with the baseline value, the mean MCV value of HIV-infected children was significantly raised at 6 months after initiation of HAART.[61]

However, the mean CD4% of HAART-experienced HIV-infected children was significantly lower than that of HAART-naïve ones. The possible reason for the low value of mean CD4% in HAART-experienced children might be delayed diagnosis and ART initiation, poor adherence to ART and delayed response to ART. These may limit the success of HAART and may be related with continued viral replication and immunological failure. Evidence also demonstrated that initiation of HAART in children with a low CD4 value is less likely to result in a robust increase in CD4 cells, thereby being less likely to achieve a successful treatment outcome.[62] Moreover, poor adherence to ART in children is one of the challenges in resource-limited settings, including Ethiopia.[63–65]

Limitations

The main limitation of this study is the cross-sectional nature of its design, which makes relationships between cytopenias and associated factors difficult, as it is temporal association. In addition, we were unable to analyze serum ferritin, vitamin B12 and folate levels and unable to perform bone marrow examination, which potentially limit this study. Another limitation of this study is that we did not assess all modifiable risk factors, such as intestinal parasitic infection, malaria, viral infections and fungal infections, which could potentially influence the magnitude and severity of cytopenias. We did

not include HIV-negative children as a control to compare the magnitude of cytopenias between HIV-infected and noninfected children. Furthermore, the study is a single-center institutional study that could not be generalized for HIV-infected children in the study area.

Conclusion

The prevalence of anemia was higher, meeting the WHO criteria for a moderate public health problem. Neutropenia was the second most common cytopenia among HIV-infected children in the study area. Severe immunosuppression and younger age were significantly associated with anemia. Moreover, the proportion of cytopenias did not significantly vary between HAART-naïve and HAART-experienced HIV-infected children. Therefore, emphasis should be given for investigation and management of hematological abnormalities in HIV-infected children, particularly those who are immunosuppressed and of younger age. Furthermore, multicentered prospective studies need to be conducted to explore modifiable associated factors of cytopenias, patterns of cytopenias over time, and the impact of HAART on cytopenia among HIV-infected children in resource-limited settings such as Ethiopia.

Abbreviations

AIDS, acquired immune deficiency syndrome; ANC, absolute neutrophil count; ART, antiretroviral treatment; CI, confidence interval; CD4, cluster of differentiation-4; ETB, Ethiopian birr; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; Hg, hemoglobin; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; mid, mixed cell; MPV, mean platelet volume; OIs, opportunistic infections; RBC, red blood cell; RDW, red cell distribution width; WBC, white blood cell

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Author contributions

YGT participated in conceiving and designing the study; collecting, analyzing and interpreting the data as well as drafting the manuscript. MM participated in conceiving and designing the study; analyzing and interpreting the data and drafting the manuscript, in addition to being the lead author of the manuscript. ZA, AT and AA participated in study design and data analysis, as well as contributing toward drafting and review of the manuscript. All authors have read and approved the final manuscript.

Disclosure

The authors report no conflicts of interest in this work.

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