

ORIGINAL ARTICLE

Prevalence of hematological abnormalities and malnutrition in HIV-infected under five children in Enugu

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Abstract

Background: Hematological abnormalities such as anemia, neutropenia, and thrombocytopenia occur in children infected by the human immunodeficiency virus (HIV). These abnormalities are due to myelosuppression caused by the HIV and contribute to the morbidity and mortality of HIV-infected children. Malnutrition is prominent in HIV-infected children due to associated conditions such as oropharyngeal candidiasis, diarrhea, and cytokine production which result in poor intake, nutrient loss, and increased metabolic rate, respectively.

Objectives: To determine the prevalence of hematological abnormalities (using the World Health Organization (WHO) case definitions) and malnutrition in HIV-infected children receiving care at the University of Nigeria Teaching Hospital, Enugu.

Materials and Methods: The hematological and anthropometric indices of HIV-infected children between 18 and 59 months were assessed. Their hemoglobin level, neutrophil, and platelet counts were the hematological profiles evaluated using the WHO case definitions in HIV clinical staging. The weight-for-height z-score index was used to assess the nutritional status of subjects using the WHO reference ranges. The *t*-test, Chi-square, and Pearson correlation coefficient were used for statistical analysis.

Results: There were 67 HIV positive children: 34 males and 33 females, aged 18-59 months. The mean hematological levels of subjects were hemoglobin (Hb) 10.4 ± 1.2 g/dl, neutrophil count $3,031 \pm 1,039$ cells/mm³, platelets count $294 \pm 78 \times 10^9/L$. Two children (3.0%) had anemia (hemoglobin < 8 gm/dl) and were severely immunosuppressed, on highly active antiretroviral therapy treatment and had advanced HIV disease (clinical stage 3). Children who were malnourished were 15 (22.4%).

Conclusion: Hematological abnormalities and malnutrition occur in HIV positive children.

Key words: Haematological, malnutrition, anaemia, children

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Introduction

The human immunodeficiency virus (HIV) infection accounts for more than 36% rise in under-five mortality in Africa.^[1] Enugu state, had a seroprevalence rate of 5.8% as of 2008.^[2]

Hematological abnormalities such as anemia, neutropenia, and thrombocytopenia occur in children infected by

HIV^[1,3,4] and the etiology is multifactorial.^[4] HIV infection causes elaboration of cytokines, depressing hemopoiesis, and resulting in hematological abnormalities.^[5] Some opportunistic infections such as tuberculosis^[6,7] and some antiretroviral drugs like zidovudine, are known to cause

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anemia due to myelosuppression.^[3,5-7] Ruhinda *et al.*,^[8] in Uganda found anemia to be prevalent in 57.6% children aged 3 months-18 years with HIV infection. Bachou and colleagues^[9] documented 8.0% prevalence for severe anemia in children below 60 months of age. It has been noted that certain conditions prevalent in HIV-infected children, such as micronutrient malnutrition (iron and folate deficiencies), determine to a large extent, the prevalence of anemia particularly.^[1] Childhood malnutrition among HIV-infected children is due to decreased food intake, increased nutrient loss from malabsorption, and diarrhea and also increased metabolic rate.^[1] In the study by Bachou *et al.*, 43.0% of the children below 60 months of age had edematous malnutrition.^[9]

There is yet no available published work on hematological abnormalities and malnutrition in HIV positive children in Nigeria. This study therefore was conducted to determine the prevalence of hematological abnormalities and malnutrition in HIV positive children aged between 18 and 59 months.

Materials and Methods

Study center and design

The study was conducted at the Pediatric Infectious Disease Clinic of the University of Nigeria Teaching Hospital. The clinic was established in 2005 to cater primarily for children with HIV exposure and infection. Children seen in the clinic are from referrals from the other clinics within and outside the hospital. This was a cross-sectional descriptive study in which subjects were recruited and enrolled by convenient sampling method on having given informed written consent and meeting the inclusion criteria for the study. The sample size of 67 children was used. This was determined with the size formula (in a descriptive study) for a proportion for population size of less than 10,000.^[10]

Subject selection

For a participant's enrolment the following criteria were used: Age between 18 and 59 months, diagnosis of HIV with ELISA test and confirmation by Western blot (at the time of this study, our center does not carry out DNA polymerase chain reaction for early infant diagnosis). The use of antiretroviral medications, cotrimoxazole and presence of opportunistic infections such as pneumocystis jiroveci pneumonia (PCP) and tuberculosis were not exclusion criteria.

Methodology

On presentation to the clinic, data on gender, age, highly active antiretroviral therapy (HAART) use and duration, duration of HIV diagnosis, and CD4 cell count and percent were obtained. The physical examination findings of the subjects were also documented. The

weight and the height of the subjects were measured using a combined measuring instrument Health Weighing Scale and Stadiometer (RTZ-120A, HECOS, China) with respective sensitivities of 0.1 kg and 0.1 cm. The weighing scales were standardized each day of the study using known weights to ensure consistent and accurate measurements. About 3 ml of subject's blood were collected in ethylenediaminetetraacetic acid (EDTA) bottles and analyzed for full blood count (FBC) using Sysmex Haematolog Analyzer, (Europe, serial number A8206). The blood samples were analyzed within 24 h of collection and were not refrigerated.

The data obtained were used to categorize the subjects. Advanced HIV disease was defined as WHO clinical stage 3 and 4, while stage 1 and 2 defined nonadvanced HIV disease.^[1] Duration of HIV diagnosis is the time from diagnosis of HIV in the subject to the time of the study. Using the WHO stratification for age-related CD4 values,^[1] the HIV-positive under-five children, were stratified into two different age groups; 18-35 months (Group 1) and 36-59 months (Group 2). Severe immunosuppression was defined as CD4% of less than 25% for Group 1 and CD4% of less than 20% for Group 2.^[11] The anthropometric values of weight and height were used to classify the nutritional status of the children using the World Health Organization (WHO) weight-for-height z-score charts.^[12] Values from above-1 standard deviation (SD) to + 1SD defined well-nourished children while malnutrition was defined as the index-1SD and below.^[13] Weight-for-height z-score less than-1SD to-2SD was mild malnutrition, below-2SD to-3SD was moderate malnutrition, while values below-3SD was severe malnutrition.^[13] Using the WHO case definition of hematological indices,^[11] anemia was defined as hemoglobin concentration of less than 8 g/dl, neutropenia was absolute neutrophil count of less than 1,000/mm³ and thrombocytopenia was platelet count of less than 50,000/mm³ ($50 \times 10^9/l$).^[11]

Ethical approval was obtained from Health Research and Ethics Committee of University of Nigeria Teaching Hospital (UNTH), Enugu before commencing the study. The subjects' data were safely stored and the confidentiality of information obtained was secured.

Demographic and laboratory data were analyzed using the Statistical Package for Social Sciences (SPSS) version 15.0 for Windows® (IBM Inc, Chicago Illinois, USA). Continuous variables such as the age, duration of HAART, and hematological indices were expressed as mean and standard deviations, median, and range. Comparison of means of continuous variables was done using *t*-test and analysis of variance (ANOVA). For categorical variables, Chi-squared test (Fisher's exact test, if indicated) was used for test of significance. To ascertain relationships between hemoglobin levels and subjects' characteristics, Pearson

correlation coefficient was used for continuous variables. Significant levels were set with $P < 0.05$.

Results

A total of 67 children were enrolled. There were 34 males, with a male: female ratio of 1:1. The median age of the study population was 48 months (range of 27-59 months), while the mean duration of HIV diagnosis and HAART treatment were 13.9 ± 10.4 (95% CI, 11.3-16.4, $P < 0.0001$) and 10.1 ± 10.0 months (95% CI, 7.6-12.6, $P \leq 0.0001$), respectively. Majority (71.6%, 48/67) was on HAART, Table 1 and the HAART regimen included zidovudine for all those on HAART. All the children were on cotrimoxazole prophylaxis. The mean values of the hematological indices are shown in Table 2.

Only two subjects (all females) had anemia with equal occurrence in each age groups (Fisher's exact = 0.27, $P = 1.000$), giving a prevalence of anemia in HIV positive children as 3.0%, (2/67). The anemic subjects were older (mean age = 47.0 ± 17.0 months, 95% CI, 105.5-199.5) than those without anemia (mean age = 44.3 ± 9.5 months, 95% CI 41.9-46.6), $P = 0.697$. The mean CD4% of anemic subjects was 11.5 ± 2.1 (95% CI, -7.2 to 30.4) compared to those without anemia 22.3 ± 9.6 (95% CI, 19.9-24.7), $P = 0.121$. The two subjects who had anemia were severely immunosuppressed, had advanced HIV disease were on HAART and malnourished [Table 3]. The remaining 46 children on HAART were not anemic. None of the subjects had neutropenia or thrombocytopenia. However, there was comparatively low neutrophil count in those on HAART treatment, $t = 0.662$, $P = 0.062$; Table 3. A direct

relationship was demonstrated between CD4% and hemoglobin level; $r = 0.40$, $P = 0.001$ [Table 4].

Fifteen of the children (22.4%, 15/67) were malnourished, and there were more males than females and more of the older age group; $\chi^2 = 0.796$, $P = 0.851$; $\chi^2 = 5.455$, $P = 0.141$; respectively [Table 5]. Of this number, seven were on HAART ($\chi^2 = 5.934$, $P = 0.015$), two had advanced HIV disease (Fisher's exact = 7.147, $P = 0.047$), two were anemic (Fisher's exact = 7.147, $P = 0.047$), and six were severely immunosuppressed ($\chi^2 = 2.578$, $P = 0.108$). The malnourished children had shorter duration of HIV diagnosis and HAART treatment than well-nourished children [Table 6]. There was a direct correlation between the weight-for-height z-score and age of subjects, duration of HIV diagnosis, and duration of HAART [Table 7].

Discussion

The only hematological abnormality seen in this study was anemia with a prevalence rate of 3.0%. Anemia has been documented by several studies^[1,3,4,8,9,14,15] as a common hematological abnormality in HIV-infected children. Contrary to the low prevalence rate in this study, Adetifa et al.,^[3] Ira^[4] with their colleagues found the prevalence of anemia to be 77.9 and 70%, respectively. Bachou et al.,^[9] documented 8.0% prevalence for severe anemia. This marked difference could be from the varied definitions ascribed to anemia by the different studies; whereas, the index study used WHO case definition for anemia as documented in WHO clinical staging system for HIV: Hb < 8 g/dl, in HIV positive children.^[11] Adetifa and colleagues defined it as Hb < 10 g/dl,^[3] while Ira and Bhushan used Hb < 11 g/dl.^[4] These^[3,4] were higher values

Table 1: General characteristics of the study population

Parameters	WHO clinical staging								Total
	Stage 1		Stage 2		Stage 3		Stage 4		
	M*	F**	M	F	M	F	M	F	
Medication									
On HAART									
18-35 months	4	3	1	2	0	1	0	0	48 (71.6)
36-59 months	14	13	6	3	0	1	0	0	
Not on HAART									
18-35 months	5	4	1	1	0	0	0	0	19 (28.4)
36-59 months	2	4	1	1	0	0	0	0	
Total	49 (73.1)		16 (23.9)		2 (3.0)		0 (0)		67 (100)
Immunosuppression									
Severe									
CD4% <25 for 18-35 months	2	2	1	2	0	1	0	0	24 (35.8)
CD4% <20 for 36-59 months	6	5	2	2	0	1	0	0	
Non severe									
CD4% ≥25 for 18-35 months	7	5	1	1	0	0	0	0	43 (64.2)
CD4% ≥20 for 36-59 months	10	12	5	2	0	0	0	0	

*M=Male, **F=Female, HIV=Human immunodeficiencyvirus, HAART=Highly active antiretroviral therapy

Table 2: Hematological indices of the study population

Indices	Parameters	
	Mean \pm SD	95% CI
Hemoglobin g/dl	10.4 \pm 1.2	10.1-10.7
Neutrophil count in cells/mm ³	3,031 \pm 1,039	2,778-3,284
Platelet count $\times 10^9/l$	294 \pm 78	275-313

SD=Standard deviation, CI=Confidence interval

Table 3: Subject's characteristics and hematological indices

Parameters	Mean Hb \pm SD in g/dl	Mean neutrophil count \pm SD in cells/mm ³	Mean platelet count \pm SD in 10 ⁹ /L
Medication			
On HAART	10.2 \pm 1.2	2883.1 \pm 988.2	289.9 \pm 82.4
Not on HAART	11.1 \pm 1.2	3406.6 \pm 1095.7	303.4 \pm 68.1
<i>t</i> (P value)	0.857 (0.003)	0.662 (0.062)	0.292 (0.530)
Disease severity			
Advanced HIV disease	7.4 \pm 0.1	2917.0 \pm 1538.7	378.5 \pm 101.1
Nonadvanced HIV disease	10.5 \pm 1.1	3035.1 \pm 1037.2	291.1 \pm 77.1
<i>t</i> (P value)	0.056 (< 0.001)	0.546 (0.876)	0.696 (0.121)
Immunosuppression			
Severe	9.9 \pm 1.4	3104.2 \pm 912.2	269.3 \pm 88.9
Nonsevere	10.7 \pm 1.0	2991.0 \pm 1111.7	307.4 \pm 69.2
<i>t</i> (P value)	0.204 (0.006)	0.230 (0.672)	0.245 (0.056)
Nutritional status			
Well-nourished	10.5 \pm 1.1	3016.5 \pm 1008.6	294.3 \pm 71.9
Malnourished	10.3 \pm 1.6	3083.5 \pm 1173.3	291.8 \pm 100.5
<i>t</i> (P value)	0.066 (0.716)	0.687 (0.828)	0.148 (0.913)

HIV=Human immunodeficiency virus, HAART=Highly active antiretroviral therapy, SD=Standard deviation

Table 4: Correlation between haemoglobin level and age of subjects, duration of HIV infection, duration of HAART treatment, weight-for-height of subjects, and CD4%

Parameters	Pearson correlation (r)	P value
Age of subjects in months	-0.068	0.583
Duration of HIV infection in months	-0.083	0.506
Duration of HAART treatment in months	-0.125	0.315
Weight-for-height of subjects	-0.088	0.477
CD4%	0.390	0.001

HIV=Human immunodeficiency virus, HAART=Highly active antiretroviral therapy

representing moderate and mild anemia, respectively^[16] and may have contributed to higher prevalence rates. However, Bachou *et al.*, used Hb < 5 g/dl for severe anemia in HAART naïve children below 60 months of age.^[9]

The high CD4 cell count of our subjects may have contributed to the low prevalence of anemia, as HIV positive children with high CD4 cell count are less likely to have anemia.^[7,14] Mata-Marín and others^[17] in their study

Table 5: Nutritional abnormalities by age and gender

Parameters	Weight for height			Total	
	Well-nourished (%)	Malnourished (%)			
		Mild	Moderate	Severe	
Age range					
18-35 months	15 (28.8)	6	0	1	22
36-59 months	37 (71.2)	4	3	1	45
Total	52		15		67
Prevalence of malnutrition (%)				22.4	
χ^2 (P value)				5.455 (0.141)	
Gender					
Male	25 (48.1)	6	2	1	34
Female	27 (51.9)	4	1	1	33
Total	52		15		67
χ^2 (P value)				0.796 (0.851)	

Table 6: Nutritional status and characteristics of the study population

Mean parameters	Well-nourished subjects	Malnourished subjects	<i>t</i>	P value
Age in months	45.0 \pm 9.2	42.1 \pm 11.0	0.165	0.299
Duration of HIV diagnosis in months	14.9 \pm 10.9	10.5 \pm 7.7	0.034	0.151
Duration of use of HAART in months	11.7 \pm 10.2	4.4 \pm 7.2	0.012	0.011
Hb in g/dl	10.5 \pm 1.1	10.3 \pm 1.6	0.066	0.716
CD4%	22.3 \pm 9.8	21.0 \pm 9.6	0.748	0.663

HIV=Human immunodeficiency virus, HAART=Highly active antiretroviral therapy

Table 7: Correlation between weight-for-height value and age of subjects, duration of HIV infection, duration of HAART treatment, hematological indices of subjects, and CD4%

Parameters	Pearson correlation (r)	P value
Age of subjects in months	0.293	0.016
Duration of HIV diagnosis in months	0.460	<0.001
Duration of HAART treatment in months	0.477	<0.001
Haemoglobin level of subjects in g/dl	-0.088	0.477
Neutrophil count of subjects in cells/mm ³	-0.069	0.579
Platelet count of subjects in 10 ⁹ /l	0.209	0.090
CD4%	0.095	0.445

HIV=Human immunodeficiency virus, HAART=Highly active antiretroviral therapy

documented CD4 cell count below 200 cells/mm³ (severe immunosuppression) as the only risk factor associated with anemia in their subjects. In the study by Ruhinda *et al.*,^[8] advanced disease, low CD4 cell count young age (age range of study population 3 months-18 years) were risk factors associated with anemia. Interestingly, our subjects with anemia were all severely immunosuppressed and had advanced HIV disease. One of the etiologies of anemia in HIV infection is suppression of erythropoiesis by

cytokines which are produced by HIV-infected lymphocytes, monocytes, and macrophages.^[6] In advanced HIV disease, there is corresponding increase in viral load and decrease in number and function of CD4 cells.^[18] The increase in viral load results in elaborated production of cytokines by the increased number of HIV-infected lymphocytes, monocytes, and macrophages. This may explain why HIV-infected children with anemia were in advanced stage of the disease and severely immunosuppressed.

The study subjects with anemia were receiving zidovudine in their HAART combination therapy. Although treatment with HAART improves anemia in most HIV positive children,^[8] Zidovudine based HAART has been shown to be a risk factor for the development of anemia in HIV positive children as it causes myelosuppression.^[5,7,14] Okechukwu and coworkers^[7] found that children on zidovudine based HAART were at higher risk for anemia than those on stavudine based HAART. Zidovudine intake is also an independent predictor of persistent anemia in those who had anemia prior to commencement of zidovudine based HAART.^[5] Some studies^[3,4,9] documented high prevalence of anemia in HAART naïve HIV positive children, thus highlighting the depressant effect of HIV on hemopoiesis.^[19] Furthermore, we found that those with anemia had shorter duration of HAART treatment compared to those without anemia.

This study demonstrated that malnutrition occurs in HIV positive children as did the study by Bachou *et al.*,^[9] who documented 43.0% prevalence of edematous malnutrition in HAART naïve children. The use of HAART has been shown to improve the nutritional indices of HIV positive children.^[20] In this study, 47% of those who were malnourished were not on HAART treatment and the remaining half on treatment had a significant shorter duration of treatment. The effect of HAART on nutritional status could be attributed to a reduction in viral load and subsequent decrease in body metabolic rate that usually accompanies infectious processes. It is also pertinent to note that there was no demonstrable association between nutritional status and Hb level in this study, which is similar to the finding by Ira and Bhushan.^[4] This could be because the anthropometric indices used in calculating nutritional status in that^[4] and the index study assess macronutrient deficiency, whereas deficiencies of micronutrients such as iron, vitamin B complex, and folic acid deficiencies are factors associated with anemia.^[17]

We did not find severe neutropenia or thrombocytopenia in our study, although malnourished subjects and those on HAART had comparatively low neutrophil and platelet counts. Myelosuppression consequent on HAART treatment and malnutrition could have contributed to this finding. However, in a study on populations who were HAART naïve, Ira and Bhushan^[4] found the prevalence rates of 2 and

10%, respectively for neutropenia and thrombocytopenia. In addition to their subjects being HAART naïve, they^[4] defined neutropenia as absolute neutrophil count less than 1,500 mm³ and thrombocytopenia as platelet count less than 150,000 mm³, which could explain the observed prevalence rates.

As been documented, myelosuppression in HIV-infected children can result from zidovudine based HAART regimen^[5,7,14] or from overwhelming cytokines in absence of HAART treatment.^[6] Therefore, the presence or absence of hematological abnormalities in these children will depend on the balance between the myelosuppression and other risk factors such a micronutrient deficiency.

Conclusion

Hematological abnormalities and malnutrition occur in HIV positive children. Anemia is a common hematological abnormality and has a direct relationship between CD4 cell counts. The older child and those with longer duration of HIV diagnosis and HAART treatment were more malnourished. Further study, on a larger population of children, to ascertain the role of other factors, such as malaria and micronutrient deficiency, which may contribute to anemia and malnutrition in HIV positive children, is recommended.

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