ORIGINAL ARTICLE

HEMATOLOGIC ABNORMALITIES AMONG CHILDREN ON HAART, IN JIMMA UNIVERSITY SPECIALIZED HOSPITAL, SOUTHWESTERN ETHIOPIA

Muluneh Abebe, MD, Fessahaye Alemseged, MD,MPHE

ABSTRACT

BACKGROUND: ***In individuals infected with human immunodeficiency virus, hematological abnormalities are associated with increased risk of disease progression and death. However, the magnitude and severity of hematological abnormalities in those patients who are taking antiretroviral drugs is not known in Ethiopia. Hence this study was conducted to determine the magnitude and severity of anemia, neutropenia and thrombocytopenia in HIV infected children who are taking highly active antiretroviral therapy in Jimma University Specialized Hospital.***
METHODS: ***A cross-sectional study was conducted from August to November, 2007 on 64 HIV infected children who have been taking highly active antiretroviral therapy for more than two months in the study hospital. Data were collected using structured questionnaire that included variables related to socio-demographic characteristics, immunohematological profiles and clinical conditions of the study individuals. Data was analyzed using SPSS for Windows version 12.0.1.***
RESULT: ***The prevalence of anemia, thrombocytopenia and neutropenia among the study children was 21.9%, 7.8% and 4.7%, respectively. Severe life threatening anemia was seen in 2(14.3% of the child). The mean level of hemoglobin, thrombocyte count and CD4 count showed statistically significant increment from the baseline (p-value <0.05)***.
CONCLUSION: ***Hematologic abnormalities were common problems among the children taking highly active antiretroviral therapy. Therefore, clinicians need to routinely investigate and treat hematological abnormalities before and after treatment and furthermore large scale and longitudinal studies are recommended to strengthen and explore the problem in depth.***

KEYWORDS: ***Anemia, Thrombocytopenia, Neutropenia, HIV, Highly Active Antiretroviral Therapy, Zidovudine***

INTRODUCTION

Acquired Immunodeficiency Syndrome (AIDS) is one of the most destructive epidemics in the history of mankind. In Ethiopia the adult prevalence of HIV was estimated to be 2.2% in 2008. The total number of People Living with HIV/AIDS (PLHIV) in the same period was estimated to be 1,037,267 adults and 68,136 of them were children. Furthermore the number of deaths due to AIDS for the same period was estimated to be 58,290 for adults and 9,284 among children (1).

In HIV infected individuals hematological abnormalities are common and they increase the risk of morbidity and mortality. In both antiretroviral-treated and untreated individuals, anemia is independently associated with an increased risk of disease progression and death (2-4).

Although HAART is known to profoundly suppress viral replication, it increases CD4 cell count and delays disease progression and death; patients on Highly Active Antiretroviral Therapy (HAART) commonly suffer from side effects of the drug (5-7). Each antiretroviral drug is associated with specific adverse effects. Among the antiretroviral drugs, Zidovudine (AZT) remains to be the most widely used drug resulting in myelosuppression (6, 8).

Several studies in developed countries have shown that AZT alone and AZT based HAART regimen is associated with significant reduction of hemoglobin (Hb) level and neutrophil number (3, 7, and 8). Though most of the studies on hematological abnormalities are on adults, one randomized comparative trial done to assess the safety and efficacy of AZT and d4T in symptomatic HIV infected children showed a prevalence of anemia to be 5% among the AZT group whereas 2% among the d4T group. Similarly the prevalence of neutropenia was higher in AZT group (9).

[1]Department of pediatrics and Children Health, Jimma University
[2]Department of Epidemiology & Biostatistics, Jimma University

Although 25.8 million people are living with HIV/AIDS in Sub Saharan Africa, few studies tried to assess the safety and efficacy of HAART. In one multicentered study conducted in Uganda, Kenya and Zambia 12% of patients on AZT-based HAART regimen switched drug because of drug related severe anemia or GI toxicity (10).

At the end of February, 2007, 72,127 HIV patients were started on Antiretroviral Therapy (ART) at 234 facilities across the country (11). Regardless of the fact that Ethiopians' normal immunohematologic profile is known to be lower than the white population by 2 to 3% (12), AZT-based HAART is one of the first line regimens in the guideline (13).

The impact of HAART on the hematological profile of Ethiopian HIV/AIDS patients is not known. Hence this research was conducted to determine the magnitude of hematological abnormalities and the possible associated factors.

METHODS

This cross-sectional study was conducted from August 1 to November 10, 2007 at Jimma University Specialized Hospital (JUSH), Southwest of Ethiopia which is a teaching and referral hospital for about 15 million people. The hospital has ART center that provides voluntary counseling and testing, care and treatment for HIV infected children and adults. At the time of the study there were 2,166 PLWHA on follow-up care, of which 997 were adults and 68 children were on ART (13). Sixty four of the 68 children who took HAART for more than two months were included in the study.

Data were collected using structured pre-tested questionnaire. The questionnaire had three parts, the first part for collecting data about socio-demographic characteristics of the study subjects, the second part for collecting data concerning baseline characteristics of the individuals before initiation of HAART mainly clinical, laboratory and immunological characteristics and the third part for collecting data related to HAART treatment and the change in baseline parameters after taking HAART. The respondents were parents or immediate caregivers of the study children.

Data were collected by two nurses who had training certificate and experience in care and treatment of patients with HIV. Laboratory measurements were done by experienced laboratory technologist. One medical doctor worked as supervisor of the whole process. All study subjects were approached during their respective appointment schedule for follow up. After interview and detailed review of the medical record, the study subjects were sent to laboratory where blood was collected for determination of complete blood count and CD4 count, and the result was collected with in six hours and filled on the data collection format. To ensure the quality of data, training was given to data collectors and supervisors and pre-test was done on adult patients. Besides, the collected data were checked for completeness and internal consistency.

Data were coded, cleaned, entered and analyzed using SPSS for Windows version 12.0.1. Descriptive analysis was done to assess the prevalence of anemia, neutropenia and thrombocytopenia. Bivariate and multivariate analyses were done to look for association between the presence of anemia and independent variables.

Anemia, neutropenia and thrombocytopenia were defined based on World Health Organization (WHO) grading of hematologic toxicity (14). Accordingly, anemia was defined as Hb concentration less than or equal to 10g/dl for all children and further severity was classified into grades as follows: Hb level of 8.5 – 10 g/dl as Grade 1; 7.5 - <8.5 g/dl as Grade 2; 6.5 - < 7.5 g/dl as Grade 3; and < 6.5 g/dl as Grade 4. Grade 3 and 4 are further labeled as severe life threatening anemia. Neutropenia was defined as absolute neutrophil count (ANC) of less than 1000 /mm^3 and based on Pediatric Adult Clinical Trial Group (PACTG) classification, severity was further classified as follows: Grade 1: 750 - <1000 /mm^3; Grade 2: 500 - 749 /mm^3; Grade 3: 250 – 499 /mm^3 and Grade 4: < 250 /mm^3. Platelet count between 150,000 and 450,000/mm^3 was classified as normal, greater than 450,000/mm^3 as thrombocytosis and less than 150,000/ mm^3 as thrombocytopenia.

Ethical clearance was obtained from Jimma University (JU) ethical committee and permission was obtained from hospital authorities to conduct the study. After informing parents/caregivers about the objective of the study and the confidentiality of the data, consent was obtained. To ensure confidentiality of data, study subjects were identified using codes and unauthorized persons did not have access to the collected data. Furthermore, the hematological findings were utilized for proper management of the patients.

RESULTS

Of the 68 children, 64 who have been taking HAART for more than two months were included in the study. The mean age of the study subjects was 6.9 (3.2) years. The male to female ratio was 1.13:1. With regard to the survival status of their parents, majority, 50(78.1%) of the study children had lost one or both of their parents. Concerning the caregivers' educational status, 15(23.4 %) were illiterate, 22(34.4%) were elementary and 27(42.2%) were high school and above. 36(56.3%) of the caregivers were unemployed and 42 (65.6%) earned monthly income below 300 Birr (Table 1).

Table 1. Socio-demographic Characteristics of HIV Infected Children on HAART at JUSH, 2007.

Demography	N (%) (n=64)
Sex	
Male	34(53.1)
Female	30(46.9)
Age in years	
< 3	5(7.8)
3-5	22(34.4)
6-8	15(23.4)
9-13	22(34.4)
Religion	
Muslim	27(42.2)
Orthodox	33(51.6)
Protestant	4(6.3)
Ethnicity	
Oromo	34(53.1)
Amhara	21(32.8)
Kefa	3(4.7)
Dauro	5(7.8)
Gurage	1(1.6)
Parent status	
Both alive	14(21.9)
Father alive	13(20.3)
Mother alive	18(28.1)
Neither alive	19(29.7)
Family income	
< or = 300	42(65.6)
301-600	10(15.6)
601+	12(18.8)
Educational Status of Care givers	
Illiterate	15(23.4)
Grade 1-6	22(34.4)
Grade 7-12	22(34.4)
Grade12 plus	5(7.8)
Employment Status of Care Givers	
Unemployed	36(56.3)
Employed	28(43.8)
Child Knows HIV status	
Yes	22(34.4)
No	42(65.6)

With regard to the type and duration of HAART regimen, 62(96.9%) were taking AZT based HAART and 48(75%) took treatment for a duration of more than 6 months. According to WHO clinical staging 5(7.9%) of the study participants were in stage 1 and 2 while 59(92.2%) of the study participants had advanced clinical stage (stage 3 and 4). Of the study children 36(56.3%) and 27(42.2%) had severe immune suppression (CD_4 percentage below 15%) before initiation of HAART and after initiation of HAART, respectively (Table 2).

46(71.9%) and 47(73.4%) were underweight and stunted before initiation of HAART whereas, after initiation of HAART, the proportion of underweight and stunted children decreased to 27(42.2%) and 39(60.9%), respectively. The decrements were statistically significant (p-value <0.001). Before initiation of HAART, majority 57(89.1%) had history of Opportunistic Infections (OI), the commonest being Tuberculosis (TB) seen in 36(56.2%), While after initiation of HAART the prevalence of OI was 51.5% and pneumonia being the commonest 23(34.4%) (Table 2).

The mean Hb concentration increased from baseline 10.5 gm/dl to 11.2 gm/dl after treatment with HAART.

Total leukocyte count (TLC), thrombocyte, and CD_4 count and percent also showed increment. Whereas ANC and CD8 values showed some decrement in their mean values. The increments of hemoglobin, thrombocyte count and CD4 count were statistically significant (p-value <0.05) (Table 3).

Table 2 . Clinical and Immunological Characteristics of HIV Infected Children on HAART at JUSH, 2007.

Variable	Before (n=64) N (%)	After HAART (n=64) N (%)	P-value
CD4 lymphocyte %			
Less than 15%	36(56.3)	27(42.2)	0.00
15-24%	10(15.6)	20(31.3)	
Greater than 25%	14(21.9)	17(26.6)	
Wt for Age			
< 5th percentile	46(71.9)	27(42.2)	0.00
> or 5th Percentile	18(28.1)	37(57.8)	
Ht for Age			
< 5th percentile	47(73.4)	39(60.9)	0.00
> or = 5th percentile	17(26.6)	25(39.1)	
Wt for Ht			
<70 percent	1(1.6)	0	0.08
70 – 79 percent	4(6.3)	3(4.7)	
80 – 89 percent	20(31.3)	11(17.2)	
> or = 90 percent	39(60.9)	50(78.1)	
Opportunistic infections			
Chronic GE	4(6.3)	0	
Pneumonia	13(20.3)	21(32.8)	0.01
PCP	2(3.1)	3(4.7)	
TB	34(53.1)	6(9.4)	
TB & Pneumonia	2(3.1)	0	
PCP & Pneumonia	2(3.1)	1(1.6)	
No OI	7(10.9)	33(48.5)	

Hematological abnormalities were present both before and after treatment with HAART. Anemia (Hb ≤10gm/dl) was found in 34(53.1%) of subjects before and in 14(21.9%) of the subjects after initiation of HAART. Among the anemic cases, 5(14.7%) and 2(14.3%) had severe life threatening anemia before and after initiation of HAART, respectively (Table 4). Out of the 34 anemic patients at baseline 20 (58.8%) improved from anemia.

Neutropenia was seen in 5(7.8%) and 3(4.7%), thrombocytosis in 3(4.7%) and 9(14.1%), thrombocytopenia in 12(18.8%) and 5(7.8%) of the children before and after initiation of HAART, respectively (Table 4).

Table 3. Mean Hematological values of HIV Infected Children, before and after Initiation of HAART at JUSH, 2007.

Variables	Hematological Values		
	Before HAART Mean (SD) (n=64)	After HAART Mean (SD) (n=64)	P-value
Hemoglobin (Hb)	10.5 (2.4)	11.2 (1.9)	0.04
AZT based	10.6 (2.5)	11.2 (2.0)	
D4T based	8.5 (0.7)	12 (0.0)	
WBC x 10^3	7.0 (3.0)	6.9 (2.6)	0.79
TLC x 10^3	2.6 (1.6)	3.0 (1.5)	0.08
ANC x 10^3	3.7 (2.4)	3.2 (1.5)	0.12
Thrombocyte x 10^3	264 (139)	318 (132)	0.01
CD4	384 (356)	519 (371)	0.00
CD8	1368 (718)	1340 (709)	0.76

After controlling confounding effect, children who were below age five years (OR=2), male (OR=1.6), stunted (OR=1.3), underweight (OR=4.1), and having CD4 count <25% after highly active antiretroviral treatment (OR=6.1), were more likely to have anemia as compared to their counter parts. However, none of the associations were statistically (Table 5).

Table 4. Patterns and Severity of Hematological Abnormalities in HIV Infected Children before and after Initiation of HAART at JUSH, 2007**.**

Variable	Before (n=64) N (%)	After(n=64) N (%)	P value
Hb			
Hb > 10gm/dl	30(46.9)	50(78.1)	0.12
Hb < or = 10gm/dl	34(53.1)	14(21.9)	
Anemia severity			
Grade 1(8.5-10.0)	23(67.7)	9(64.3)	*NA
Grade 2 (7.5-8.4)	6(17.6)	3(21.4)	
Grade 3(6.5-7.4)	4(11.8)	0	
Grade 4(<6.5)	1(2.9)	2(14.3)	
Neutrophil count	N=59	N=61	
> or = 1000	54(84.4)	58(90.6)	*NA
< 1000	5(7.8)	3(4.7)	
Neutropenia Severity			
Grade 1(750-999)	3(4.7)	3(4.6%)	*NA
Grade 2(500-749)	2(3.1)	0	
Grade 3(250-499)	0	0	
Grade 4(< 250)	0	0	
Thrombocyte	N=57	N=62	
>450 x 10^3	3(4.7)	9(14.1)	
150-450 x 10^3	42(65.6)	48(75.0)	*NA
<150 x 10^3	12(18.8)	5(7.8)	

*NA (not applicable) as chi-square requirements are not met.

DISCUSSION

In Ethiopia, there is lack of baseline data on HIV or HIV treatment related hematological abnormalities. The findings of this study hence partially fill the gap. However, the small number of study population limits the generalizability of the results.

The prevalence of anemia before initiation of treatment in this study was consistent with reports of other studies that ranged from 36.9% to 95% depending on the stage of the disease (14). Compared to the finding of review of 32,867 adult and adolescent HIV patient medical records that showed a prevalence rate of 36.9%, our finding is higher. This difference could be attributed to the higher prevalence of anemia in the local context and possibly due to the difference in age and size of the study population (3, 15).

After initiation of HAART therapy the mean Hb level increased by 0.7mg/dl from baseline. On further analysis, the increment in the mean Hb concentration was higher in those patients who have been taking d_4T than AZT based HAART (3.5 g/dl Vs 0.6 g/dl). When we compare the mean change of Hb with the meta-analysis of the six prospective, randomized controlled trials which showed a decrement by 0.4g/dl at 6 months and 0.2g/dl at 12 months in AZT group but an increment by 0.45g/dl at 6 months and 0.58g/dl at 12 months in d4T group, our finding showed higher increment in both regimens (3). This difference could again be due to relatively low baseline prevalence of anemia in the latter study which is conducted in developed country and partly due to the difference in study design and size of study populations.

Neutropenia was also seen both before and after initiation of treatment. The prevalence of Neutropenia before treatment (7.8%) was higher than after treatment (4.7%). All cases of neutropenia were patients who were taking AZT based HAART. As compared to the meta-analysis which reported 26-46% neutropenia in AZT recipients and the prospective randomized comparative trial of d_4T and AZT in children which found neutropenia of 20% over one year among AZT recipients (8, 9) our finding is lower. However, the small size of the study population in this study could limit making a valid comparison between the findings.

Table 5. Coefficients and OR from logistic regression model predicting the probability of Anemia in HIV Infected Children on HAART at JUSH, 2007.

Variables	ß coefficient	Crude OR (95% CI)	Adjusted OR (95% CI)
Age			
≤5 years		1.7(0.49, 6.27)	2.0 (0.48, 8.63)
> 5 years	0.714	1	1
Sex			
Male	0.453	1.8 (0.528, 6.23)	1.6 (0.39, 6.38)
Female		1	1
Current			
Weight for age			
< 5 percentile		4.9 (1.32, 17.78)	4.1 (0.72, 23.05)
> or = 5 percentile	1.404	1	1
Height for age			
< 5 percentile		2.9 (0.71, 11.60)	1.3 (0.20, 8.92)
> or = 5 percentile	0.282	1	1
Duration of HAART treatment			
≤ 6 months			
> 6 months		1	1.3 (0.29, 5.65)
	0.256	1(0.26, 3.61)	1
CD4 % after HAART treatment			
< 25%		6.1 (0.735, 50.914)	6.1 (0.67, 56.38)
> or = 25%	1.812	1	1

Thrombocytosis and thrombocytopenia were also found before and after treatment with HAART. The prevalence of thrombocytopenia after treatment (7.8%) was similar with finding of the randomized comparative trial of AZT and d_4T in children which showed 7% (3).

In addition to the hematological abnormalities, this study has also given us some insight about the efficacy of

HAART which was demonstrated by improved weight for age from the baseline, decreased rate of opportunistic infection, increased mean CD4 value and CD4 lymphocyte percentage.

In conclusion in this study; anemia, neutropenia, thrombocytopenia and thrombocytosis were common both before and after HAART treatment among the study subjects. Nonetheless, HAART resulted in increment of the mean Hb concentration irrespective of the regimen used. Based on these findings it is recommended that physicians giving care for HIV infected children should routinely investigate and treat hematological abnormalities before and after treatment. Additionally large scale and longitudinal studies are recommended to strengthen and explore the problem in depth.

ACKNOWLEDGEMENTS

We would like to acknowledge Jimma University for funding the study. We also like to express our thanks for all of the study participants.

REFERENCES

1. UNAIDS, December 2007 Report.
2. Mocroft A, Kirk O and Barton S. Anemia is an independent predictive marker for clinical prognosis in HIV-infected patients from across Europe. EuroSIDA study group. AIDS, 1999; 13:943-50.
3. Sullivan S, Hanson L, Chu Y, Jeffrey J and John W. Epidemiology of anemia in human immunodeficiency virus (HIV) infected persons: Results from multistate Adult and Adolescent Spectrum of HIV Disease Surveillance Project. Blood. 1998; 91: 301-308.
4. Ludwig H and Strasser K. Symptomatology of Anemia. Semin Oncol. 2001; 28: 7-14.
5. D'Arminio A, Lepri C and Rezza G. Insights into the reasons for discontinuation of the first highly active antiretroviral therapy (HAART) regimen in a cohort of antiretroviral naïve patients. I.CO.N.A. Study Group. Italian Cohort of Antiretroviral-Naïve Patients. AIDS, 2000; 14:499-507.
6. De Jesus E, Herrera G and Teofilo E. Abacavir versus zidovudine combined with lamivudine and efavirenz, for the treatment of antiretroviral-naive HIV-infected adults. Clinical Infectious Disease, 2004; 39:1038-46.
7. Eron J, Yetzer S and Ruane J. Efficacy, safety, and adherence with a twice-daily combination lamivudine/zidovudine tablet formulation, plus a protease inhibitor, in HIV infection. AIDS, 2000; 14:671-81.
8. Moyle G, Sawyer W, Law M, Amin J and Hill A. Changes in the Efficacy of Thymidine Analogue-based HAART: A Meta-analysis of Six Prospective, Randomized, Comparative Studies. Clinical Therapeutics, 2004; 26:1.
9. Mark W, Russel B, Jane C, et al. A Randomized trial of Stavudine versus Zidovudine in Children with Human Immunodeficiency Virus Infection. Pediatrics 1998; 101: 214-220.
10. Amoroso A, Sheneberger R, Edozien A et al. Antiretroviral-associated Drug Toxicities Leading to a Switch in Medication: Experience in Uganda, Kenya and Zambia. 14th Conference on Retroviruses and Opportunistic Infections Los Angeles. California Feb 25- 28, 2007.
11. Ministry of Health of Ethiopia – HAAPCO. Monthly HIV Care ART Update released on March 22, 2007.
12. Kassu A, Tsegaye A, Petros B, et al. Distribution of Lymphocyte Subsets in Healthy Human Immunodeficiency Virus-Negative Adult Ethiopians from Two Geographic Locales. Clinical and Diagnostic Laboratory Immunology. Nov. 2001, p. 1171–1176.
13. Ministry of Health of Ethiopia. Guideline for Pediatric HIV/AIDS Care and Treatment in Ethiopia. Addis Ababa, July 2007.
14. Moyle G. Anemia in Persons with HIV Infection: Prognostic Marker and Contributor to Morbidity. AIDS Rev, 2002; 4:13-20.
15. Adish A, Esrey S, Gyorgis T and John T. Risk factors for iron deficiency anemia in preschool children in northern Ethiopia. Public Health Nutrition. 1999; 2(3): 243-252.