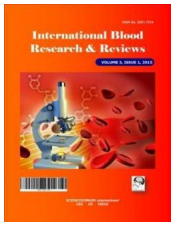

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Anti-retroviral Treatment Related Haematological Disorders among HIV- Infected Children Attending HIV Clinic at Yekatit 12 Hospital, Addis Ababa, Ethiopia

Mestewat Debasu[1], M. K. C. Menon[2*], Yididya Belayneh[2], Workeabeba Abebe[3], Degu Jerene[4] and Daniel Seifu[2]

[1]Department of Biochemistry, St. Paul's Hospital Millennium Medical College, P.O.Box 1271, Addis Ababa, Ethiopia.
[2]Department of Medical Biochemistry, School of Medicine, Faculty of Health Sciences, Addis Ababa University, P.O.Box 9086, Addis Ababa, Ethiopia.
[3]Department of Pediatrics and Child Health, School of Medicine, Faculty of Health Sciences, Addis Ababa University, P.O.Box 1176, Addis Ababa, Ethiopia.
[4]Department of Preventive Medicine, School of Medicine, Faculty of Health Sciences, Addis Ababa University, P.O.Box 1176, Addis Ababa, Ethiopia.

Authors' contributions

This work was carried out in collaboration between all authors. Authors MD, MKCM, YB, WA, DJ and DS designed the study, wrote the protocol, the first and subsequent drafts of the manuscript. Author MD performed the analytical and experimental process and author DS provided the laboratory facilities and administrative support. All authors read and approved the final manuscript

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ABSTRACT

Background: The Management of drug toxicities is increasingly becoming a crucial component of human immunodeficiency virus infection and improvement of antiretroviral therapy in developing countries like Ethiopia. The severity of hematological abnormalities in children who are taking ART is not known well in Ethiopian Hospitals.

**Corresponding author: E-mail: menakathmenon@gmail.com;*

Objective: The study was undertaken to determine the severity of HAART related hematological disorders in HIV positive children who were on HAART at Yekatit 12 Hospital, Addis Ababa, Ethiopia.
Methods: A cross sectional study was conducted from May 2012 to February 2013 among children who had been on HAART for maximum of twelve months. Data collection using questionnaires and measurement of complete blood count and CD4 + T cell counts were made by using standard methodology. The results were tested using appropriate statistical methods (mean, Standard Deviation, Odd Ratio, p-value and F test value).
Results: A total of 106 patients (<18 years) were enrolled in the study and had a mean age of 6.5±3.4 years, a median age of 7 years and female to male ratio of 1.04:1. The prevalence of anemia was 18.9%, 12.3% and 10.4% at baseline, at six months, and at twelve months post-treatment, respectively. Their mean hemoglobin level increased by 1.0 gm/dl at six months and by 1.7 gm/dl at twelve months of follow up with statistically significant values ($p < 0.001$), and F test value presented 15.87. Patients who were put on AZT based regimen were more likely to develop anemia than those on D4T-based regimen, (OR=4.5, p-value <0.05). The prevalence of thrombocytopenia at baseline was 8.5%, but it was lowered for both at six and twelve months by 5.7%. The thrombocyte count of AZT based regimen showed statistically significant association (p-<0.05) and F test value as 2.98. The prevalence of neutropenia at baseline and at six months was similar with the value of 2.8% while at twelve months it was higher at 5.7%.
Conclusion: Anemia, neutropenia and thrombocytopenia were the hematological abnormalities among HIV infected Ethiopian children taking HAART. Anemia was the most common abnormality, but significantly lesser than in many other hospitals in Ethiopia and among those who were on AZT based regimen. It is recommended that even in limited laboratory monitoring, HAART can be safely used and health professionals may consider other risk factors associated with the development of cytopenia before selection of second line of HAART drug regimen.

Keywords: HAART; anemia; neutropenia; thrombocytopenia; Ethiopian children.

1. INTRODUCTION

In human immunodeficiency virus (HIV) infected individuals hematological abnormalities are common and they increase the risk of morbidity and mortality. In both antiretroviral treated and untreated HIV-individuals, cytopenia is independently associated with an increased risk of disease progression and death. Although highly active anti-retroviral therapy (HAART) is known to profoundly suppress viral replication, by increasing cluster of differentiation 4 (CD4) cell count and delays disease progression; patients on HAART commonly suffer from side effects of the drug. Each antiretroviral drug is associated with specific adverse effects. Among the antiretroviral drugs, Zidovudine (ZDV) formerly Azido thymidine (AZT) remains to be the most widely used drug resulting in myelosuppression [1]. Several studies in developed countries have shown that ZDV alone and ZDV based HAART regimen is associated with significant reduction of hemoglobin (Hgb) level and neutrophil number. Although 25.8 million people are living with HIV/AIDS in Sub Saharan Africa, only few studies tried to assess the safety and efficacy of HAART. In one multicenter study conducted in Uganda, Kenya and Zambia 12% of patients on ZDV based HAART regimen switched drugs because of drug related severe anemia or GI toxicity [1,2].

Globally, there were 3.4 million children living with HIV in 2010, which accounts 390,000 new infections among children, and 250,000 AIDS deaths. By the end of 2008, of the 33.4 million people living with HIV/AIDS worldwide, 15.7 million were women and 2.1 million were children under 15 years of age. In 2009, there were approximately 16.6 million AIDS orphans (children who have lost one or both parents to HIV), most of whom live in sub-Saharan Africa (89%). Additionally, an estimated 2.5 million children were living with HIV at the end of 2009 with 92% in sub-Saharan Africa [3].

In HIV infected infants and children before initiation of ART, many factors need to be considered, including potency of the ART, resistance testing and potential for future sequencing, palatability, available formulations and dosing recommendations, disclosure and adherence issues, and potential drug-drug interactions [4].

Despite the fact, most new HIV infection among children is through vertical transmission (mother to child transmission) (MTCT). HIV positive

women pass the virus to the baby during pregnancy, during delivery or by breast feeding. It is estimated that over 90% of children were infected with HIV in utero, during the delivery or breast feeding. Intrapartum transmissions are mediated by direct contact of infant mucosa with HIV-laden maternal blood, amniotic fluid, and cervical/vaginal secretions [5-6]. In Ethiopia, about 1.1 million people are living with HIV, out of which the children constituted about 72000, which is a worrying situation for the Health authorities [7], since most of the infection were vertically transmitted. The roll-out of antiretroviral therapy in Ethiopia has benefited the children, but the spread of the disease vertically through mother-to child transmission, and its control is the major priority in this population.

There are fewer data on ART toxicity in children than in adults, and the full spectrum of ART toxicities observed in adults has also been reported in children. However, some toxicity is less common in children than in adults. For example, hepatotoxicity is rare in children, while others are more commonly reported in children than adult, such as rash or loss of bone density. The most common toxicities include: - Hematological abnormality, mitochondrial dysfunction, lipodystrophy, metabolic abnormalities, allergic reactions and iron deficiency due to parasitic intestinal infections [8-12].

Anemia was defined as Hgb concentration less than or equal to 10gm/dl for all children less than 18 years and further severity was classified into grades as follows: Hgb level of 8.5–10 gm/dl as Grade 1; 7.5 - <8.5 gm/dl as Grade 2; 6.5 - <7.5 gm/dl as Grade 3; and < 6.5 gm/dl as Grade 4. Grade 3 and 4 are further labelled as severe life threatening anemia [8,1].

The incidence of first severe anemia was assessed among HIV uninfected infants in MTCT prevention trials in Botswana. Severe anemia rates were compared between three groups. The first group was infants exposed to maternal HAART in utero and during breast feeding (BF) and one month of postnatal AZT (HAART-BF). The second group was infants exposed to maternal AZT alone for short term in utero, six months of postnatal AZT, and breastfeeding (AZT-BF) and the third group were infants exposed to maternal short-term AZT alone in utero, one month of postnatal AZT, and formula-feeding (AZT-FF). A total of 1719 infants 691 HAART-BF, 503 AZT-BF, and 525 AZT-FF were analysed Severe anemia was detected in 118 infants (7.4%). By six months, 12.5% of HAART-BF infants experienced severe anemia, compared with 5.3% of AZT-BF and 2.5% of AZT-FF infants, from this figure, the result obtained for AZT-FF infants were surprisingly low, since AZT has one of the most notorious side effects expressed as anemia. It is important to note that there was no comparison with a group whose mothers were not given any ART during pregnancy [13]. One randomized comparative trial study done to assess the safety and efficacy of AZT and 2',3'-didehydro-2',3'-dideoxythymidine (D4T) or Stavudine in symptomatic HIV infected children showed a prevalence of anemia to be 5% among the AZT group and may occur within 4–6 weeks whereas 2% among the D4T group [14,1]. In a study conducted at Burkina Faso, there was reduction in the frequency of mother-child transmission when mothers received ART. However, mothers CD4+ count cannot be considered as a parameter for setting a ART regimen [5].

In Ethiopia, as a result of rapid expansion of service facilities and improved awareness, the number of patients on ART rose sharply over the years. In 2003, Ethiopia lunched the fee- based ART and free ART in 2005, delivered as part of the comprehensive HIV/AIDS care [15,16]. In 2007, ART was started in many facilities across the country. AZT based HAART is one of the first line regimens in the guideline [1,17,18]. The impact of HAART on the hematological profile of Ethiopian HIV/AIDS patients and children is currently under investigation in many hospitals across Ethiopia. This research was undertaken to fill the gap in the literature about the hematological abnormalities and the associated risk factors and to evaluate it in the HIV infected Ethiopian children admitted to a urbanized hospital.

2. MATERIALS AND METHODS

2.1 Study Design

Cross sectional study design was used to assess the hematological profile among HIV/AIDS infected children who were on HAART at Yekatit 12 Hospital ART clinic, Addis Ababa. Study was conducted from March 2012 to February 2013 in Addis Ababa, ART unit of Yekatit 12 Hospital. This institution is selected based on the availability of patients from all parts of the country as it is referral and urban general specialized teaching hospital in Ethiopia as well as the ease of access and sufficient availability of the data in this unit. As of February 2013, a total

of 409 HIV infected children were on ART at Yekatit 12 Hospital. The source population for this study, was HIV positive children age less than 18 years and using ART, and attending follow up at Yekatit 12 Hospital had been considered.

The study population consisted of a total of 409 HIV infected children less than 18 years old who had started HAART (defined as taking two or more antiretroviral drugs for 12 months,), by World Health Organization (WHO) clinical and immunological criteria and who had complete blood count (CBC), CD4 count and thrombocyte counts taken at the time of HAART initiation, and also six and twelve months after initiation of the treatment.

The hospitals in Addis Ababa, Ethiopia provides to HIV- infected children both first line and second line drug regimens. An Etiologic classification on anemia is based on the various conditions that can result from any of the physiologic changes and helps determine direction for planning care [19,20]. First line regimens that children were taking included 4a = d4T/3Tc/NVP (Stavudine, Lamivudine, Nevirapine), 4b = d4T/3Tc/EFV (Stavudine, Lamivudine, Efavirenz), 4c = AZT/3Tc/NVP (Zidovudine, Lamivudine, Nevirapine), and 4d = AZT/3Tc/EFV (Zidovudine, Lamivudine, Efavirenz).

Some children in city hospitals in Addis Ababa, were also on the following second line regimens: ABC/ ddi/LPv/r (Abacavir, Didanosine, retonavir enhanced Lopinavir), AZT/3Tc/LPv/r (Zidovudine, Lamivudine, retonavir enhanced Lopinavir) and D4T/3TC/LPv/r (Stavudine, Lamivudine, retonavir enhanced Lopinavir) [7].

2.2 Study Subjects

All HIV positive children age less than 18 years and using ART for 12 months and attending follow up during the study period, who meet the inclusion criteria and who gave their informed consent and assent.

2.3 Sample Size Determination and Sampling Techniques

2.3.1 Sample size

The required sample size for this study is calculated based on the prevalence rate of 16.19% reported in previous study [21] and the 95% confidence interval and 5% marginal error, the sample size (n) is determined using the following statistical formula:

$$n = \frac{Z^2 . P\,(1 - P)}{d^2}$$

$$n = \frac{1.962 \text{ x } 0.1619(1-0.1619)}{0.0025} = 106$$

d = margin of error between the sample and the population
n = sample size
Z = 95% confident interval
P = prevalence rate of hematological disorders based on the previous study
N = 409

2.3.2 Eligibility criteria

2.3.2.1 Inclusion criteria

(1) All HIV positive Children less than 18 years old at the time of HAART initiation.
(2) Who had started ART and had follow up in Yekatit 12 Hospital, Addis Ababa
(3) Baseline (Pre-HAART), six and twelve months follow up data with complete hematological values (CBC, CD4 cells count and thrombocyte count)
(4) Who were not diagnosed as having hematological diseases of any identified cause (including hemolytic anemia, thrombocytopenia and haemoglobino-pathy) before ART initiation.
(5) Those who volunteered to participate in the study (parents or care givers were consulted, and informed consent obtained)

2.3.2.2 Exclusion criteria

(1) HIV positive children, less than 18 years old who were severely sick due to other medical conditions
(2) Those children on treatment for anemia
(3) Those using medications for either anemia, neutropenia or thrombocytopenia. Those who have any hematological abnormality before initiation of ARTs
(4) Those who were on medication (antibiotics, vitamin supplements and tuberculosis treatment) at the time of sampling.

2.4 Sampling Techniques

Non-probability sampling technique was used. HIV positive children who had enrolled at Yekatit

12 Hospital ART unit from May 2012 to February 2013 and those HIV positive children who were meeting the inclusion criteria during the study period were included in the study.

2.5 Data Collection Procedure

2.5.1 Questionnaires

A structured questionnaire was used for data collection. The questionnaire was developed in English and translated into Amharic language. 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. The third part for collecting data related to HAART treatment and the change in baseline parameters (adverse effect) after taking HAART. The respondents were parents or immediate care givers of the study children.

2.5.2 Anthropometric measurements

The assessment of growth by objective anthropometric methods such as weight and height in relation to their ages, and weight in relation to height is crucial in child care to assess the nutritional status and for the identification of growth failure. Thus, anthropometric measurement helps to diagnose under nutrition (underweight, stunted and wasting), overweight and obesity and, other growth related conditions by referring WHO growth reference charts [22,10]. Therefore anthropometric measurements were carried out according to the WHO recommendations. Nurses and supportive care givers helped with the collection of data.

2.6 Blood Specimen Examination for Hematological Assay and Immunological Assay

2.6.1 Sample collection and preparation

A volume of 5ml of venous blood was collected from each patient. The blood, 2ml and 3ml was then dispensed into two vacutainer ethylene diamine tetra acetic acid (K_2 EDTA) tubes, respectively. The 2 ml sample was used for immunological analysis (CD4 estimation) and 3ml sample was used for CBC analysis.

2.6.2 Hematological assay

2.6.2.1 Complete blood count

Complete hematological parameters white blood cell (WBC) - Differential, WBC, red blood cell (RBC) and platelet (PLT) were performed using Abbott Hematology Analyzer CELL-DYN 1800 (Abbott, USA). The CELL-DYN 1800 System is a bench-top analyzer consisting of the main analyzer with data module, display station with external printer [23].

2.6.3 Immunological assay

CD4 counts in % were assayed by BD FACS COUNT system analyzer, using % software (Becton Dickenson and Company, California, USA). Standard procedures were used for absolute CD4 T cell count [24,25].

N.B Measurements that were used for data collection procedures such as the anthropometric measurement and the hematological measurements that were done, are part of routine procedure for all patients those attending ART unit in Yekatit 12 Hospital.

2.7 Study Variables

2.7.1 Dependent variable: Status of hematologic disorders

Independent variable: It includes age, sex, nutritional status, CD4 count, opportunistic infection and WHO Clinical stage.

2.8 Ethical Approval

Ethical clearance was obtained from Research and Ethics Review Committee of the Department of Medical Biochemistry, School of Medicine, College of Health Sciences, Addis Ababa, University, Ethiopia with a protocol number of SOM/BCHM/010/2012. Detailed explanations were given about the objectives, risks, and benefits of the study to the study subjects, and parents/caregivers. Strict confidentiality of responses were maintained during the study. Data were collected after obtaining informed consent from the parent/ caregiver, and assent from the children aged 12-18 years.

2.9 Data Analysis

The data was analyzed using SPSS (16^{th} version) and expressed at 95% confidence

interval and the p-value were considered significant and very significant at p <0.05 and p ≤0.001, respectively. Then data computed using appropriate statistical methods (mean, standard deviation, p-value, odd ratio, F test statistic value and one-way repeated measures ANOVA) and the results are presented using tables and figures.

3. RESULTS

3.1 Socio-demographic Characteristics, Clinical Staging and Drug Regimen

Of the 409 HIV infected children, 106 who were on HAART for maximum of 12 months were included in the study. Their ages ranged from 3 months to 16 years with a mean for age of 6.5±3.4 years and a median age of 7 years. Majority of age groups were between 5 years to 15 years accounting 61.3%, whereas only 0.9% patient fell in the age group between 14 years to 18 years. Out of the 106 patients, 50.9% were females and 49.1% were males giving a female to male ratio of 1.04:1. The result of children's parent status indicated that in 39.6% both parents were alive and in 22.6% both parents were lost. The majority of educational status of caregivers' at the time of the study was grade 12 and above, which accounts 40.6%, whereas grade 7-12, 25.5% and illiterate, 18.9%. 34.0% of the caregivers were unemployed and 25.5% earned monthly income below ≤ 420 Birr. Among the 106, 54.7% children did not know their HIV status.

According to the duration and type of HAART regimen, all study subjects took treatment for the duration of ≥1 year. The most widely used ART regimen in this study was 4a (d4T-3TC-NVP) 45.3% followed by 4c (AZT-3TC-NVP), 25.5%.Based on WHO clinical staging, majority of the study participants were in stage III 59.4%, stage II was seen in 21.7%, stage IV was seen in 14.2% and stage I was seen, in only 4.7% of the study participants. According WHO clinical classification, stage III and stage IV are considered advanced clinical stages (Table 1).

3.2 Clinical and Immunological Characteristics

As shown in Table 2, 66% of the study children at baseline had severe immune suppression (CD4 percentage below 15%) and only 0.9% participant's had CD4 percentage above 25%. After six months of HAART initiation, 41% participants had CD4 percentage above 25% while 16% participants were above 25%. The finding was statistically significant as p - value < 0.001 indicated. The value of underweight and stunted children before the initiation of HAART was 27.4% and 36.8%, respectively. However after initiation of HAART, these figures were decreased significantly to 19.8% and 19.8% for both underweight children and stunted children, respectively. The decrements of stunted children

Table 1. Baseline Socio-demographic characteristics, WHO clinical staging and drug regimen of HIV infected children who started HAART at Yekatit 12 Hospital

Demography	N(%), (n = 106)
Sex	
Male	52 (49.1)
Female	54 (50.9)
Age in years	
< 18 months	7 (6.6)
18 – 60 months	33 (31.1)
5 -14 years	65 (61.3)
14 – 18 years	1 (.9)
Parent status	
Both alive	42(39.6)
Father alive	24(22.6)
Mother alive	16(15.1)
Neither alive	24(22.6)
Family income	
≤ 420	27(25.5)
421 - 600	14(13.2)
600^{+}	65(61.3)
Educational status of caregivers	
Illiterate	20(18.9)
1 - 6	16(15.1)
7 - 12	27(25.5)
12^{+}	43(40.6)
Employment status of care givers	
Unemployed	36(34.0)
Employed	70(66.0)
Child Knows HIV status	
Yes	48(45.3)
No	58(54.7)
***Type of ART**	
4a	48(45.3)
4b	16(15.1)
4c	27(25.5)
4d	15(14.2)
WHO Clinical stages	
Stage I	5 (4.7)
Stage II	23(21.7)
Stage III	63(59.4)
Stage IV	15(14.2)

**4a=d4T-3TC-NVP, 4b= d4T-3TC-EFV, 4c= AZT-3TC-NVP, 4d= AZT-3TC-EFV*

after HAART were statistically significant (p-value <0.001) but the value of underweight did not significant change. At baseline majority of children were in >70 of weight for height (wasting), which accounts 71.7%, and only 6.6% was in ≥ 90. At six months of HAART initiation, the value of >70 weight for height decreased to 40.6% and the value of ≥ 90 was increased to 17%. The statistic showed significant increments of weight for height, (p-value <0.001). At baseline, the majority of children, 67.9%, had history of Opportunistic Infections (OI), the commonest being recurrent pneumonia seen in 16.1%, and oral thrash was seen in 9.4%, while after the initiation of HAART, majority of children 77.4% did not have history of OI while 22.6% had OI (Table 2).

3.3 Hematological Values before and after Initiation of HAART

As shown in Table 3, the mean Hgb concentration was increased and found to be 12.2 gm/dl on base line and 13.3 gm/dl after six months of HAART. The mean Hgb concentration based on type of HAART regimen was increased in both AZT and D4T based regimen as compared to baseline, it accounts for 12.7±2.6 and13.6±2.5 respectively after treatment with HAART. The mean CD4 count in % showed increment, 13.2±5.2 at baseline and 24.1±7.8 at six months of HAART. Mean corpuscular volume (MCV), mean cell hemoglobin volume (MCH), mean cell hemoglobin concentration (MCHC) and thrombocyte count also showed increment. Whereas ANC showed some decrement in their mean values at six month, that was 3.2±1.7 but at base line it was 4.0±3.7. The increments of Hgb, MCV, MCH, MCHC and CD4 count in % showed statistically significant with p-value < 0.001 whereas thrombocyte count and ANC showed statistically significant association (p-value <0.05) (Table 3).

3.4 Patterns and Severity of Hematological Abnormalities

In the study subjects hematological abnormalities were observed both before and after treatment with HAART (Fig. 1). The study subject whose

Table 2. Clinical, immunological characteristics and anthropometric measurements of HIV infected children at baseline and after six months with HAART

Variable	Before (n=106) N (%)	After six month (n=106) N (%)	P- value
CD4 lymphocyte %			
Less than 15 %	70 (66.0)	17 (16.0)	0.000
15-24 %	35 (33.0)	45 (42.5)	
Greater than 25 %	1 (0.9)	44 (41.5)	
Weight for age			
< 5th percentile	29 (27.4)	21(19.8)	0.088
> or = 5th percentile	77 (72.6)	85(82.2)	
Height for age			
< 5th percentile	39 (36.8)	21(19.8)	0.001
>or = 5th percentile	67 (63.2)	85(80.2)	
Weight for Ht			
<70	76 (71.7)	43(40.6)	0.000
70-79	16 (15.1)	23(21.7)	
80-89	7 (6.6)	22(20.8)	
>or= 90	7 (6.6)	18(17)	
Opportunistic Infections			
Chronic GE	3(2.8)	3(2.8)	0.298
Recurrent Pneumonia	27(25.5)	9(8.5)	
*PCP	5(4.7)	1(0.9)	
PCP + pneumonia	6(5.7)	1(0.9)	
Oral thrush	10(9.4)	2(1.9)	
*Chronic GE+ Oral thrush	10(9.4)	1(0.9)	
*Others	11(10.4)	7(6.6)	
No OI	34(32.1)	82(77.4)	

***GE** = Gastro enteritis, **PCP** = Pneumocystis carinii pneumonia pneumonia*

***others**= Unexplained persistent fever, Herpes zoster, fungal nail infection, Unexplained persistent parotid enlargement, and Recurrent or chronic upper respiratory tract infections (otitis media, sinusitis, tonsillitis)*

Table 3. Mean hematological values and mean hemoglobin values between drug regimens and mean CD4% on HIV infected children before and after six months with HAART

Hematological values			
Variables	**Before HAART Mean ±SD (n=106)**	**After HAART (six months) Mean± SD (n=106)**	**p-value**
Hemoglobin (Hgb)	12.2±2.2	13.3±2.5	0.000
AZT based	12.5±2.0	12.7±2.6	
D4T based	12±2.3	13.6±2.5	
MCV	80.5±13.5	93±12.5	0.000
MCH	27±5.3	31.7±5.3	0.000
MCHC	33.2±3.7	33.9±2.4	0.000
WBC X 10^3	6.8±3.9	6.6±2.7	0.436
TLC X 10^3	2.6±1.6	2.7±1.4	0.491
ANC X 10^3	4.0±3.7	3.2±1.7	0.034
Thrombocyte X 10^3	317±153	356±136	0.026
CD4 %	13.2±5.2	24.1±7.8	0.000

Hgb concentration > 10 mg/dl had increased their number after taking six months and twelve months of taking ART it accounted 87.7% and 89.6% respectively compared to the number of subjects before HAART 81.1%. Anemia (Hgb ≤10 gm/dl) was found in 18.9% of subjects before and 12.3% at six and 10.4% at twelve months of the subjects after initiation of HAART. Among the anemic cases, the majorities were grade I, 10.4% were at baseline, 6.6% at six months and 7.5% at twelve months of HAART. This study showed that 2.8%, 0.9 and 1.9% had severe life threatening anemia before, at six and twelve months after initiation of HAART, respectively. Total anemic patients at baseline, among them the number of patients at six months 6.6% and at twelve months 8.5% of post HAART were improved from anemia. The result showed the number of anemic cases decreased.

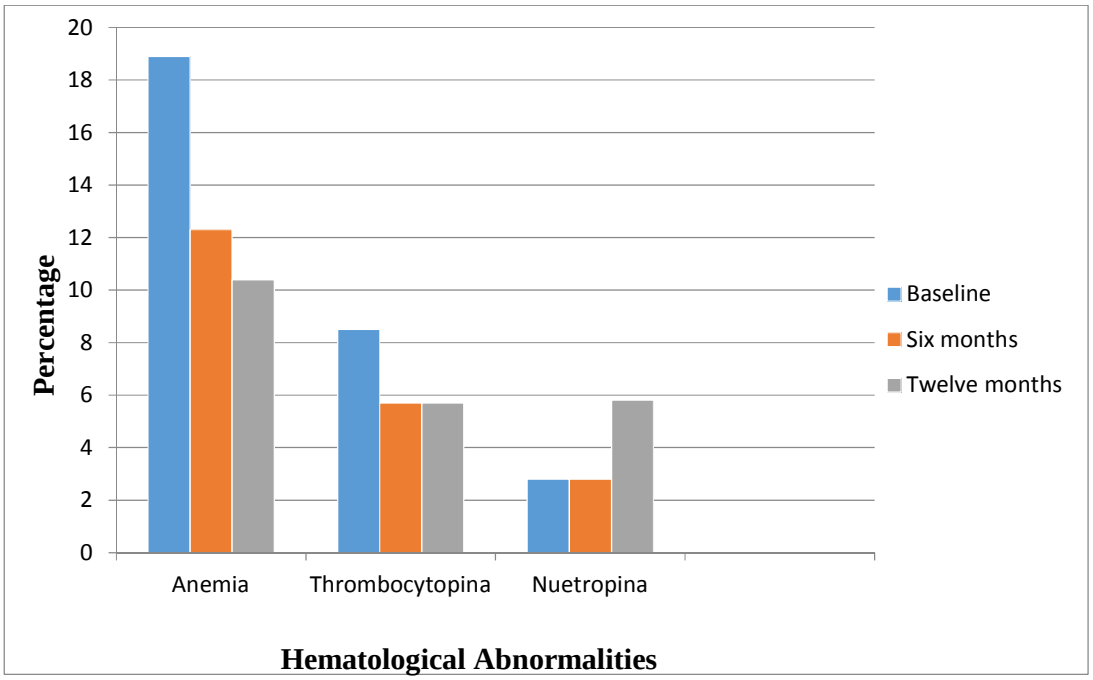

Fig. 1. Comparison of the prevalence of Hematological abnormalities (Anemia, Thrombocytopenia and Neutropenia) and its percentage at baseline, at six months and at twelve months of HAART

Neutropenia was seen in 2.8% before and after six month of HAART but 5.7% was seen at twelve months (Fig. 1). Thrombocytosis was seen for 17%, 22.6% and 19.8% at baseline, at six and twelve months respectively as well as thrombocytopenia was seen in 8.5% at baseline and twelve months where as 5.7% after six month of initiation of HAART.

The type of anemia also assessed in this study. Thus among the total number of cases, before HAART normocytic normochromic anemia was present in 45.3%, whereas after HAART it was increased to 56.6%. It was also found that before HAART, microcytic hypochromic and macrocytic normochromic anemia were present in 46.2% and 7.5%, respectively. After six months of HAART, microcytic hypochromic anemia reduced to 12.3% while macrocytic normochromic anemia was raised to present in 30.2% as shown in Fig. 2.

The result on Table 4 revealed that the study subjects were observed having mean Hgb level, neutrophil and thrombocyte count at baseline, six and twelve months of treatment with HAART. The mean Hgb level increased statistically as very significant ($p < 0.001$) and thrombocyte count increased statistically significant ($p < 0.05$), whereas neutrophil count showed decrement. At time of HAART initiation, the mean and standard deviation of Hgb concentration, neutrophil and thrombocyte count were 12.2±2.2, 13.2±2.6 and 13.7±2.2 respectively. After HAART, Hgb concentration, neutrophil and thrombocyte count were 13.2±2.6, 3.23±1712.5 and 356.3±136.4 and 13.7±2.2, 3.53±2091.7 and 340.3±149.4. Their mean Hgb level showed marked increase by 1.0 gm/dl at six month and by 1.7 gm/dl at twelve month. The observed difference is statistically very significant ($p < 0.001$) using one-way repeated measures ANOVA, F test Statistic value presented with 15.87,where as the thrombocyte count revealed statistically significant values ($p < 0.05$) and F test value was 2.98 after six and twelve month of HAART taking.

3.5 The Probability of Anemia in HIV Infected Children after Six Months of HAART

After controlling confounding effects, the probability of anemia after six months of HAART, multivariate logistic regression (Table 5) demonstrated that, 38 participant who were age

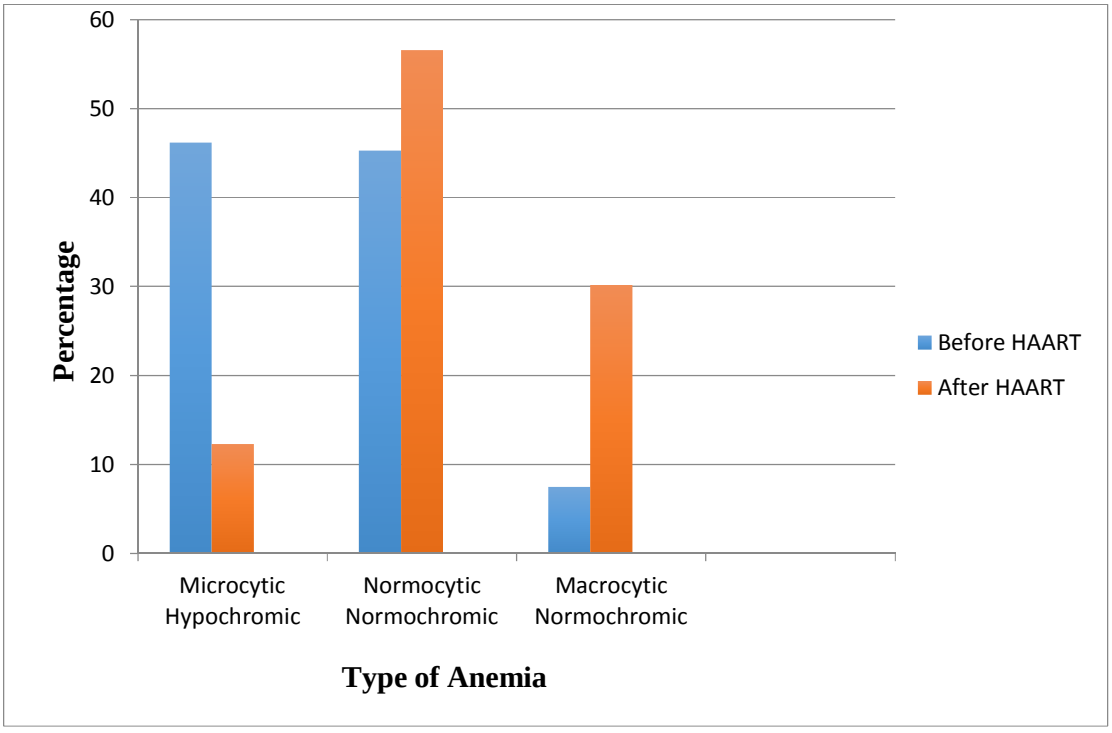

Fig. 2. Summary of type of Anemia (Microcytic Hypochromic, Normocytic Normochromic and Macrocytic Normochromic) and its Percentage of the study subjects before HAART and after HAART

Table 4. Comparison of hematological parameters (Hemoglobin, Neutrophil count and thrombocyte count) between baselines, after six months and after twelvemonths of HAART

Variable	Baseline (n=106) Mean ±SD	After six months (n=106) Mean ±SD	After twelve months (n=106) Mean ±SD	F test statistic value and P - value
Hemoglobin	12.2 ±2.2	13.2±2.6	13.7±2.2	F = 15.87, P = 0.000
Neutrophil Count	3.95±3694.5	3.23±1712.5	3.53±2091. 7	F = 2.65, p = 0.09
Thrombocyte count	316.8±153.3	356.3±136.4	340.3±149.4	F= 2.89, p = 0. 05

Table 5. Odd Ratio from logistic regression model predicting the probability of anemia in HIV infected children after six months of HAART

Variables	Anemic	Normal	Crude OR (95% CI)	Adjusted OR (95% CI)
Age				
≤ 5 years	7	31	1.88 (0.60-5.82)	3.1 (0.84-11.5)
>5 years	7	61		
Sex				
Female	6	48	0.69 (0.22-2.14)	0.83 (0.25-2.75)
Male	8	44		
Wt for age				
<5 percentile	4	17	1.77 (0.49-6.31)	2.37 (0.41-13.76)
≥ 5percentile	10	75		
Ht for age				
<5 percentile	4	17	1.77 (0.49-6.31)	1.10 (0.19-6.28)
≥ 5 percentile	10	75		
OI				
absent	12	70	1.89 (0.39-9.08)	2.03 (0.38-10.82)
present	2	22		
CD4 % after HAART Rx				
< 25%	9	53	1.33 (0.41-4.26)	3.09 (0.83-11.49)
≥ 25%	5	39		
HAART regimen Based				
AZT	9	33	3.22 (0.99-10.40)	4.54 (1.18-17.43)
D4T	5	59		

below five years, 7 were anemic (OR 3.1,95% CI (0.84-11.5), 52 male participant 8 were anemic (OR 0.83, 95% CI (0.25-2.75) and 21 study participant who were underweight among them 4 were anemic (OR 2.4, 95% CI (0.41-13.76) were independent risk factors to developed anemia. Additionally out of 21 stunted children, 4 were anemic (OR 1.1,95% CI (0.19-6.28), 62 study participant whose CD4 count < 25% among them 9 participant were anemic (OR 3.09, 95% CI (0.83-11.49) and 24 had history of OI among them 2 were anemic (OR 2.03, 95% CI (0.38-10.82) were independent risk factors to developed anemia compared to their each counter parts even if lack of association(with statistical significant) between independent risk factors. This study revealed, 42 participant who were AZT based regimen among them 9 were anemic with statistically significant association (p-value <0.05) compared to D4T based regimen (Table 5).

4. DISCUSSION

Both in infants and children, the use of antiretroviral therapy has been used safely and effectively. Younger children frequently metabolize drugs faster than adults, as food intake varies more in children and may have a greater impact on drug absorption. Growing children need frequent dose adjustments, and altered pharmacokinetic parameters need to be considered when using antiretroviral drugs safely and effectively. Therefore, in children drug dosage recommendations are meant to achieve a targeted drug exposure, usually similar to what is observed in adult patients [4,10].

In Ethiopia, there is lack of baseline data on HIV or HIV treatment related hematological abnormalities in children, in various hospitals. Therefore, the findings of this study in Yekatit 12 Hospital, Addis Ababa, Ethiopia, is an attempt to

partially fill this gap. However, the small number of patients in the present study population limits the generalizability of the results.

The main results of this study were that of the 106 HIV infected children developed hematological abnormalities at baseline, at six months and at twelve months of HAART. All study subjects were under first line of antiretroviral therapy. The hematological abnormalities include anemia, thrombocytopenia and neutropenia (Fig. 1). Among these, anemia was the most common observed abnormality in both before-HAART and after-HAART treated children. This is in accordance with studies done in Ethiopia [1,12,26], They described that hematological abnormalities were common problems among the children taking HAART. Another study done elsewhere, macrocytosis, anemia and thrombocytopenia were the commonest hematological adverse events associated with Zidovudine [27]. It is clear that HIV is accompanied by thromboembolic diseases. Supportive evidence from various laboratories indicate that, HIV is cytotonic disregulating β lymphocytes and altered release of cytokines which suppress growth of bone marrow progenitors leading to anemia [28]. Since HAART is widely available in Ethiopia, a crucial adjustment to therapy of haematological complications and even cessation of the drug regimen can be a suggestion to the medical professionals.

4.1 Prevalence of Anemia in HIV Infected Children before HAART and after HAART Initiation

In this study, anemia is the most commonly encountered hematologic abnormality in HIV patients before ART, which is in accordance with a study reported [29].This study, showed similar result in which an overall prevalence of anemia before ART initiation was 18.9%, this means that before ART initiation anemia was observed due to HIV infection and similar results, 18.9% were reported in Uganda [30]. It has been reported that 22.2% prevalence in a urban hospital in Addis Ababa and said to be much lower than the other studies done elsewhere in Ethiopia. Possibly, low prevalence of intestinal parasites in urban children has been attributed as a causative factor in lower percentage of anemic situation in this report. In a separate study at Bahir Dar at Felege Hiwot referal Hospital involving 506 HIV infected children, reported prevalence rate of anemia at baseline was 19.8% [16]. The work reported that 24.2% of the sample population was anemic at registration, but in sharp contrast with the 80% obtained in Port-Harcourt, Nigeria, amongst untreated HIV patients [31,32].

HIV patients before ART initiation had a significantly higher prevalence of anaemia (86.5%) as compared to HIV patients (80.5%) after ART initiation [29]. This study reported higher prevalence of anemia before ART initiation, whereas, the results were in line with Omoregie and colleague but not in agreement with that of Nadler and colleague in which no significant difference in the prevalence of anaemia was observed between HIV patients on HAART and their HAART naive counter parts [33,34]. The difference in anemia definition between the studies may be responsible for the difference in the results. In addition, drugs may cause myelosuppression in HIV infected patients such as antifungal agents, antiviral agents, antiretroviral, anti-Pneumocystis carinii agents and antineoplastic agents [35]. Recently, anemia was correlated with the presence of intestinal parasites/Helminths in HAART naïve patients and it was recommended that a regular check-up of the pharmokinetic data are necessary to prevent the occurrence of anemia in urban children [12].

HAART has been working as the gold standard in the management of HIV patients. This was reported to improve hemoglobin content [34]. However, it was observed that HAART did not improve Hgb content of HIV patients and [33] reports that HIV patients on HAART still develop mild to moderate anemia.

In this study the prevalence of anemia, obtained after ART initiation was 12.3% at six and 10.4% at twelve months. However, earlier reports from Ethiopia [1] states that 21.9% after six months of ART. In separate study, reported AZT based therapy was initiated in 1256 adult patients, among them 16.2% developed AZT induced anemia while 6.8% developed anemia due to D4T regimen [36] Majority of patients had anemia within six months of starting therapy in hospitals located in various regions of Ethiopia [37-41]. Anemia could be caused by the nutrient deficiencies like iron, folic acid and vitamin B12 in patients who are on HAART.

It is reported that the three commonest hematological adverse events after HAART initiation was anemia, macrocytosis and thrombocytopenia, among these anemia was present in 8.3%, of the patients [27].

It is important to note that the lower prevalence of anemia among HIV patients receiving HAART, may indicate the effectiveness of the HAART therapy in reducing viral load and improving Hgb values and it has been reported that HAART increase Hgb concentration and decreases the prevalence of anemia [42,44]. A number of mechanisms have been suggested to explain the prevalence of anemia among HIV patient on HAART. For example they include the presence of antibodies to HAART agents [34] the presence of AZT among the HAART regimen and CD4 counts [43,44,35].

4.1.1 Mean hemoglobin assessment in HIV infected children before HAART and after HAART initiation

In this study after six months and twelve months of HAART initiation the mean Hgb level increased by 1.1 mg/dl at six months and 1.5 mg/dl at twelve months of treatment from baseline. In another study conducted in Jimma, Ethiopia showed mean Hgb increment at six months by 0.7 g/dl from baseline. On further analysis, the increment in the mean Hgb concentration was higher in those patients who had been taking D4T than AZT based HAART (3.5 gm/dl Vs 0.6 gm/dl) [1]. This study revealed by further analysis, the increment in the mean Hgb concentration was higher in those patients who have been taking D4T than AZT based HAART (1.6 g/dl Vs 0.2 g/dl.).

It was also reported that by comparing the mean change of hemoglobin with the meta-analysis of the six prospective, randomized controlled trials which showed a decrement by 0.4 g/dl at six months and 0.2 g/dl at twelve months in AZT group but an increment by 0.45 g/dl at six months and 0.58 g/dl at twelve months in D4T group, our finding showed some increment in both AZT based and D4T based regimen [45].

The different results from the above study is explained due to relatively low baseline prevalence of anemia in the study which is conducted in a developed country and partly due to the difference in study design and size of study populations [46], and also due to the definition of anemia <13 g/dl for males while the females <12 gm/dl and anemia was defined as Hgb ≤ 12.5 gm/dl both for males and females and <10 gm/dl for children [47,43,1]. It is important that a unified definition of anemia, such as WHO definition, be used. The findings in some reports also state that, not all patients on HAART had AZT in their regimen as was the case in this study. This is important as AZT has been reported by several authors to cause anemia by inhibition of Hbg synthesis and toxicity to bone marrow cells, particularly, erythroid lines [33,43,44].

4.1.2 Risk factors for the development of anemia in HIV infected children both before HAART and after HAART initiation

The risk factors for anemia in HIV infected children are multifactorial. Among those AZT related anemia reported in various studies, include age, gender, advanced HIV stage (WHO stage III and stage IV), low baseline CD4 counts, opportunistic infections, low body weight and low body height. In this study, it was found that only descriptive association of these factors exist, but did not find any statistical significant association with, age, gender, advanced HIV stage, low baseline CD4 count, opportunistic infections, low body weight, low body height, which may be attributed by the small sample size. Our results support the data presented in [36], that anemia did not show any statistically significant association with low body weight, low CD4 count, WHO clinical staging.

In Some studies done elsewhere described that the prevalence of AZT associated anemia is about 10% at six months of ART; the pathophysiologic mechanism being bone marrow toxicity [47]. Similarly study done in Ethiopia, the prevalence of AZT associated anemia was found to be 36.2%. This indicates that AZT associated anemia is a significant problem causing morbidity and increasing treatment cost. The risk of developing AZT associated anemia is found to be higher in children started ART at WHO clinical stage III [48]. These differences can be attributed to different study design and use of different methodologies pertaining to inclusion and exclusion criteria.

A number of reports illustrated that the independent CD4 count is related to viral load, and it has been stated that CD4 count is a predictor of anemia [43,33,35]. However, the value for CD4 count differs and that CD4 count < 50 cells/μl is a significant predictor of anemia. A CD4 count of < 200 cells/μl was the value associated with anemia. Further studies are needed to resolve the effect of CD4 count on the prevalence of anemia.

This study shows post HAART treated male was more in possibility for the development of anemia than females. However, there was no statistical significance associated between them. However, among HAART naive HIV patients, males had significantly higher prevalence of anemia than their female counterparts. In another study reported by female gender had been reported as a risk factor for anemia among HIV patients [33,43]. The findings in this study differ most probably due to the difference in the modes of defining of anemia. Also, as the study subjects were adults, it might be due to large attribution of menstrual blood loss and drains on iron stores that occur with pregnancy and delivery [35].

4.1.3 Type of anemia before HAART and after HAART initiation

When assessed the type of anemia among the total number of cases, before HAART normocytic normochromic anemia was present 45.3%, whereas after HAART it was 56.6%. The high risk of developing the other types of anemia (normocytic hypochromic, normocytic normochromic and macrocytic normochromic) in pre-HAART patients, in this study, can be attributed to the multifactorial etiology of anemia as related to the study conducted earlier [49] where causes of anemia were associated to blood loss or decreased RBC production, increased RBC destruction and ineffective RBC production. The relatively high risk of developing microcytic hypochromic anemia found in before HAART patients as compared to those on HAART. This may reflect the overall nutritional deficiencies (malnutrition and malabsorption) associated with HIV patients. In another cross sectional study done in Ghana, the likelihood of developing microcytic hypochromic anemia in HAART-naive patients was five times more compared to those on HAART [20].

In this study as shown in Fig. 2, it was found that before HAART macrocytic normochromic anemia was present in 7.5% and after HAART macrocytic normochromic anemia present in 30.2% in before HAART and after treatment respectively. This showed that the average MCV for patients on HAART were significantly higher compared to their before HAART. Similar results were reported on macrocytosis anemia which was found in 20.6% of the patients and was one of the three commonest hematological adverse events observed after HAART initiation [27].

Elevated macrocytosis (MCV) is typically associated with vitamin B_{12} or folate deficiency and in the setting of HIV treatment reflects the use of AZT or D4T [43]. The elevated MCV observed in patients on HAART in this study could therefore be attributed to drug usage since most of them had a combination therapy of either AZT or D4T with lamivudine (3TC). Therefore, other factors will come into play considering the fact that those who were on before HAART had a similar likelihood of developing macrocytosis compared to the patients on HAART. It was also reported that low levels of vitamin B_{12} in HIV positive patients and folate deficiency was also described, in HIV infected patients [50]. Conversely, it was reported that HAART may increase serum B_{12} levels, and patients did not display characteristic findings of vitamin B_{12} deficiency, namely macrocytic anemia [51]. In another retrospective study conducted in Nigeria, the prevalence of 30.6% for macrocytic anemia associated with AZT was reported and is common in HIV-infected children [27]. Similar results (30-40%) were reported in a study done with Jamaican children [52].

It was suggested that low serum B_{12} levels are reflective of low levels of B_{12} transport proteins (transcobalamin I or haptocorrin) which are produced by neutrophil and not a tissue deficiency of B_{12}. A high percentage of neutropenia was observed in the study population with the percentages in patients on HAART being slightly higher than those who were on pre - HAART although the difference was statistically significant. Low levels of transport proteins associated with neutropenia could therefore be indirectly implicated in elevated MCV observed in both before HAART and those on HAART (AZT induced). In this study In this study however, estimations could not be conducted on serum B_{12}. vitamin levels. Therefore, HAART usage and its relationship to B_{12} vitamin levels could not be ascertained [53].

When other types of anemia were assessed amongst the total number of cases as Fig. 2 reveled that, before HAART normocytic normochromic anemia was present in 45.3%, whereas after HAART it was 56.6%. The high risk of developing the other types of anemia (normocytic hypochromic, normocytic normochromic and macrocytic hypochromic) in pre-HAART patients in this study can be attributed to the multifactorial etiology of anemia were associated to blood loss or decreased RBC production, increased RBC destruction and ineffective RBC production. However, reports concerning the relationship between CD4 count

and anemia are conflicting. In this study CD4 count and anemia were descriptively associated but not statistically significant [49].

4.2 Prevalence of Thrombocytopenia before HAART and after HAART Initiation

Thrombocytopenia was found to be present before and after treatment with HAART. This study revealed that at baseline the prevalence of thrombocytopenia was 8.5%, similar to that of 10% reported in an earlier study [32]. Whereas both after six and twelve months of ART initiation were 5.7%. [27], Thrombocytopenia was reported to be 2.8%. It was one of the three commonest hematological adverse events observed after HAART initiation. In another study [15], the prevalence of thrombocytopenia before ART initiation was 15.8% of the total 379 patients, similar to the 16.1% reported in another study [31]. Both were higher than the present study. While similar result showed after six month initiation of ART, thrombocytopenia was only in 6.6% of the patients. Yet another study reported that thrombocytopenia can occur in 20% to 33% of pediatric patients with HIV at some time during the course of their disease. HIV directly causes thrombocytopenia in most patients. In other retrospective studies [2] the prevalence of thrombocytopenia before ART initiation was 2.4%. Another cross sectional study, which was done for 64 children in Jimma University Specialized Hospital, Ethiopia, [1] the prevalence of thrombocytopenia at six months of ART initiation was 7.8% and in other study reported which also showed similar finding of the randomized comparative trial of AZT and D4T in children were 7% after treatments [46].

The difference in results seen from the present study is probably due to the difference in the study design and size of the study population. Prevalence of thrombocytopenia after HAART was 14.9% and these findings were correlated with their respective CD4 counts (Immune status). In this study, thrombocytosis was also assessed and found that in 17% at baseline, 22.6% at six months and 19.8% at twelve months of HAART initiation. In another study conducted [1], thrombocytosis was 4.7% at baseline and 14.1% at six months of ART initiation.

But in the present investigation, the results could not be correlated with the immune status of the patients. Since HIV is a prothrombotic condition, disruption of normal balance of coagulation factors, with an increase of prothrombotic proteins, that fail to normalize the disturbances, can be predicted from the present study [54].

4.3 Prevalence of Neutropenia before HAART and after HAART Initiation

Neutropenia was also observed both at baseline, at six months and at twelve months of initiation of HAART. This study revealed the prevalence of neutropenia for both at baseline and at six months of HAART was same, it accounts 2.8% whereas 5.7% at twelve months of HAART. The prevalence of neutropenia before treatment was 7.8% and after six months of treatment was 4.7% in the first reported case. [1]. Our finding differs from the above report, as it is lower before treatment and higher after treatment. As compared to the meta-analysis which reported 26-46% neutropenia in AZT recipients and the prospective randomized comparative trial of D4T and AZT in children which found neutropenia of 20% over one year among AZT recipients [43]. Our observation shows that it is lower at twelve months of treatment that was 5.7 %. However these differences are explained by the differences in the study population and the study design. A valid comparison is not warranted from these findings [55].

In addition to the hematological abnormalities, this study has also given us some insight about the efficacy of HAART and to ensure the maximum level of safety when delivering ART. This was demonstrated from the anthropometric data collected by improved weight for age, height for age, weight for height from the baseline, decreased rate of opportunistic infection and increased mean CD4 percentage [56].

5. CONCLUSION

Anemia, thrombocytopenia, thrombocytosis and neutropenia were the hematological abnormality encountered in children with HIV/AIDS and who are on highly active antiretroviral therapy. Among those a resulted in increment of the mean hemoglobin concentration irrespective of the regimen used. Hemoglobin level showed significant changes at six months for AZT based regimen, while absolute neutrophil count and thrombocyte count showed statically significant change at twelve months.Accordingly, early diagnosis and appropriate treatment of anemia, thrombocytopenia and neutropenia both before initiation and after initiation of HAART improves the quality of life for HIV-infected children.

AZT drug regimen was a risk factor for anemia. While, other risk factors such as children whose age less than five years, male gender, CD4 count < 25%, the presence of opportunistic infections and advanced WHO clinical staging was associated with the development of anaemia. It can be concluded that before HAART microcytic hypochromic anemia was common whereas, after HAART macrocytic normochromic anemia was common. It is suggested that clinical management of these diseases needs to be adjusted delicately to suit the haematinic deficiencies.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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