HEMATOLOGIC ABORMALITIES AMONG ADULT HUMAN IMMUNODEFICENCY VIRUS-INFECTED PATIENTS IN JIMMA UNIVERSITY SPECIALIZED HOSPITAL AT ANTI RETROVIRAL THERAPHY FOLLOW UP CLINIC , JIMMA, SOUTH WEST ETHIOPIA

BY TAMIRAT EDIE (MD)

A RESARCH REPORT TO BE SUBMITTED TO DEPARTMENT OF INTERNAL MEDICINE, COLLEGE OF PUBLIC HEALTH AND MEDICAL SCIENCE OF JIMMA UNIVERSITY, FOR PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE CERTIFICATE IN INTERNAL MEDICINE SPECIALITY

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Abstract

Background: In individuals infected with HIV, hematological abnormalities are common and are associated with increased risk of disease progression and death. However, the profile of hematological abnormalities in HIV infected adult patients is not known in Jimma university specialized hospital.

Objective: The aim of this study was to assess the hematological manifestations of HIV infection and to identify the factors associated with cytopoenias in both HAART naïve and HAART patients at JUSH ART follow-up clinic.

Method: A cross-sectional hospital based survey was conducted from July to August 2012 on 361 HAART and HAART naïve HIV infected adult patients. Patients from each category were included in the study consecutively until a quota proportionate to the size for each category was obtained. Data were coded, entered, cleared and analyzed using SPSS version 20. Descriptive, bivariate and multivariate analyses were done. Statistical significance was declared at $p<0.05$ with 95%CI.

RESULT : The prevalence of anemia, leucopenia, thrombocytopenia and lymphopoenia among the study individuals were 51.5%, 13%, 11.1% and 5% respectively. In multivariate logistic regression analysis presence of opportunistic infection (AOR 5.72, 95% CI 2.05-15.97; p=0.001), use of CPT (AOR 1.65, 95% CI 1.02-2.67; p=0.04) and CD4 count <200 cells/µl (AOR 3.34, 95% CI 1.57-7.1; p=0.002) were associated with an increased risk of anemia. And CD4 count <200 cells/µl (AOR 3.34, 95% CI 1.59-7.02; p=0.001) and use of CPT (AOR 2.34, 95%CI 1.05-5.19; p=0.036) were associated with an increased risk of leucopenia.

CONCLUSION: Hematologic abnormalities were common in HIV infected adult patients. Of the cytopoenias anemia was the most common. Use of CPT was independently associated with increased risk of anemia and leucopenia. Therefore, large scale and longitudinal studies; giving emphasis on association of CPT and cytopoenia; are recommended to strengthen and explore the problem in depth.

KEY WORDS: Anemia, Leucopenia, Lymphopoenia, Thrombocytopenia, HIV, HAART, CPT

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Chapter I: Introduction

1.1- Background information

AIDS was first recognized in 1981 as a disease caused by the HIV virus. Since then it is estimated that about 40 million people have been infected by HIV and 5 million new HIV infections occur every year making the disease HIV/AIDS, a major health problem.

In 2008, an estimated 33.4 million people were living with HIV/AIDS worldwide; nearly 70% of these were found in sub-Saharan Africa. In Ethiopia the adult prevalence of HIV was estimated to be 2.2% in 2008. The total number of People Living with HIV/AIDS (PLWHA) in the same period was estimated to be 1,037,267 of which 68,136 were children (1).

Altered hematopoiesis (blood cell production) occurs in patients with HIV infection. This change affects all three cell lines (red blood cells, white blood cells, and platelets) and Consequently, HIV-infected patients may suffer from anemia, leucopenia, thrombocytopenia, or any combination of these three. They are common throughout the course of HIV infection and may be the direct result of HIV ;at the stem cell level or the mature blood cell level**;** manifestations of secondary infections and neoplasms, or side effects of therapy. The use of cART could positively or negatively affect these parameters, depending on the choice of combination used. But generally it will reverse most of the complications that are the direct result of HIV infection. (2, 3, 4)

Anemia is the most common hematologic abnormality in HIV-infected patients. The causes of HIV-related anemia are multifactorial and HIV infection may lead to anemia in many ways: changes in cytokine production with subsequent effects on hematopoiesis, decreased erythropoietin concentrations, opportunistic infectious agents such as *Mycobacterium avium* complex or parvovirus B-19, and administration of chemotherapeutic agents such as zidovudine or ganciclovir. Less common mechanisms for HIV-associated anemia include vitamin B_{12} deficiency and autoimmune destruction of red blood cells. Direct infection of marrow precursor cells by the virus has been hypothesized, but not proven. Anemia is a treatable condition in HIV infection and all reversible causes of anemia should be treated using standard approaches, such as treating opportunistic infections, nutritional supplementations (eg, iron, folate, vitamin B12),

discontinuation of an offending drug or use of an Erythropoiesis-stimulating agent. More over studies have shown that Initiation of HAART reduces both the incidence and degree of anemia. (2, 4, 5, 6, 7, 8, 9, 10)

Neutrophils are a type of white blood cells that mainly attack bacteria and fungi. During the course of HIV infection, neutropenia may be seen in approximately half of patients. In most instances it is mild; however, it can be severe and can put patients at risk of spontaneous bacterial infections and diseases that are not commonly seen in HIV-infected patients, such as aspergillosis or mucormycosis, may occur. It can result from: impaired hematopoiesis directly or indirectly by HIV virus, other opportunistic infections, such as cytomegalovirus and *Mycobacterium avium* complex, causing myelo-suppression and drug Hematologic toxicities, such as zidovudine, cidofovir, foscarnet, ganciclovir, and trimethoprim/sulfamethoxazole. The commonest cause of neutropenia is the result of drugs such as Zidovudine (AZT), the anti-CMV drug ganciclovir, or drugs used to treat cancers and tumors. Neutropenia can be treated by reducing the dose or stopping the drug which is responsible or Alternatively, if the Neutrophil count falls very low (below 500) treatment with G-CSF to stimulate the bone marrow production of white blood cells may reduce neutropenia and thus indirectly, the risk of infections.(11,12,13)

Platelets are small blood cells that are very important for blood coagulation, blood clot formation and wound healing. Thrombocytopenia is a common finding in individuals infected with HIV and the degree is generally mild to moderate; however, severe reduction of platelet count below 50,000/µL also occurs. In the current era of combination antiretroviral therapy, it is more commonly encountered among patients with uncontrolled HIV replication and hepatitis C co-infection. The causes of thrombocytopenia in HIV-infected patients can be divided into two groups; primary HIV-associated thrombocytopenia and secondary thrombocytopenia. Of which PHAT is the most common cause; clinically it resembles classic ITP. Its pathogenesis is not well understood and studies have shown that both ineffective platelet production and increased peripheral destruction are contributory and Zidovudine (AZT) has been the mainstay of therapy of PHAT. Secondary causes of thrombocytopenia are generally the result of underlying opportunistic infections, malignancy, co-morbid conditions resulting in hypersplenism, medication-associated and Thrombotic thrombocytopenic purpura-hemolytic uremic syndrome (TTP-HUS) which is rare. (14, 15, 16)

This study on hematologic abnormalities among adult HIV-infected patients is to be conducted in JUSH ART follow up clinic, as hematologic manifestations of the HIV infection are a well-recognized complication of the disease and may be clinically important.

1.2- Statement of the problem

HIV/AIDS is characterized by progressive damage to the body's immune system which results in a number of opportunistic infections, immunological and hematological complications. [17]

Hematological abnormalities are among the most common complications of HIV and have been documented to be the second most common cause of morbidity and mortality in HIV patients [18, 19] and are generally marked with cytopoenias such as anemia, neutropenia, lymphopoenia and thrombocytopenia with thrombocytopenia typically having an earlier onset than anemia and neutropenia. [20].The incidence and severity of these cytopoenias generally correlate to the stage of the disease [21, 22].

Anemia is the most commonly encountered hematologic abnormality, occurring in approximately 30% of patients with asymptomatic HIV and in as many as 75% to 80% of those with clinical AIDS; making it more common than thrombocytopenia or leucopenia in patients with AIDS.[21-24] Its prevalence is significantly higher among HAART naive patients than those on HAART .[25] Anemia is associated with progression to AIDS[26] , shorter survival times ,[27] and it is a predictor of poorer prognosis for HIV infected patients independent of the CD4 count.[28] As to different studies, Incidence of anemia is associated with: black race, female sex ,MCV <80 fl, clinical AIDS ,CD4 count <200/ μL, HIV-1 viral load ≥50,000/ mL , Use of AZT in past six months, prescription of ganciclovir, and fluconazole .[29,30]

As to different studies done to assess the patterns of anemia in HIV-infected patients; based on the Red cell morphology; Microcytic hypo chromic and Normochromic Normocytic anemias are the most frequent patterns of anemia seen in these patients. [31, 32]

Despite the facts that; the incidence of anemia is high in HIV infected patients, is associated with progression to AIDS , shorter survival times and it is a treatable condition in the majority; it often undiagnosed and untreated because it seems like an insignificant problem in the many complications that happen in HIV disease.

Thrombocytopenia and leucopenia are the other cytopoenias seen in patients with HIV/AIDS which occur less commonly than anemia. [33, 34]

Leucopenia and neutropenia and/or lymphopoenia often accompany HIV, and their prevalence increases from asymptomatic HIV-infected individuals to individuals with AIDS. During the course of HIV infection, neutropenia may be seen in approximately half of patients. It is common in the advanced stages of AIDS and in patients with low CD4 count. Its commonest causes are drugs such as AZT, the anti-CMV drug ganciclovir, or drugs used to treat cancers and tumors. [35, 36, 37]

Despite having the facts that; neutropenia is a relatively common complication in HIV infected patients, it may put patients at risk of spontaneous bacterial infections especially in those with lower CD4+ T-cell counts and it's commonest causes are drugs routinely used to treat the HIV infection or it's related complications and accordingly can be managed by reducing the dose or stopping the drug; it often undiagnosed and untreated.

Thrombocytopenia is a common finding in individuals infected with HIV, affecting approximately 40% of pts during the course of their illness and it may present at any time during the course of HIV infection, from asymptomatic infection to advanced AIDS. [38, 39] It is more frequent in patients with AIDS (21.2%) than in patients with asymptomatic HIV-infection (9.2%).[40,41] While some studies indicated that thrombocytopenia is correlated with low CD4 cell count, other studies showed no significant correlation with CD4 counts. [42, 43] Studies also showed that AZT can rapidly increase platelet counts in patients with HIV-related thrombocytopenia. (36, 37)

Although it is known that; thrombocytopenia is a frequent complication of HIV infection, it is well responsive to AZT; and AZT containing regimens have dual purpose in thrombocytopenic HIV patients; HIV-infected patients are not investigated for thrombocytopenia at time of considering HAART initiation.

Although these hematologic abnormalities can be seen in both pre ART patients and in patients on ART; a study done to assess the impact of HAART on hematologic manifestations, had shown that cytopoenias reversed in the majority of patients (thrombocytopenia, 100%; neutropenia, 91.1%; and anemia, 84.6%) after HAART initiation.[44]

Despite the fact that hematologic manifestations of HIV infection are a well-recognized complication of the disease and it increases progression to AIDS; there is no a study done on its magnitude in JUSH. Conducting this study in JUSH HIV/AIDS adult patients will have paramount importance. Hence this research aims to determine the magnitude and possible associated factors of hematological abnormalities among HIV-infected adult patients in JUSH.

Chapter II-Literature Review

The Hematologic manifestations of the HIV infection are a well-recognized complication of the disease and may be clinically important in many patients. [45, 46] And in HIV-infected patients Cytopoenia occurs frequently, especially during advanced stages of the disease. [47]

A-Prevalence of cytopoenias in HIV/AIDS

In 2011 a non-randomized cross sectional observational study conducted in eastern India in 150 HAART naïve HIV patients; the most common hematological abnormality was anemia, present in 74.7% cases, followed by leucopenia, thrombocytopenia and Pancytopenia in 38%, 23.33%, and 16% of cases respectively. [48]

Another cross-sectional study conducted in India in 2009 on Profile of hematological abnormalities in 200 HIV infected individuals of whom 66 were receiving ART and 134 were not, the overall mean age was 36.6±8.7years; Anemia was still the most common presentation and it was found in 65.5% of individuals while thrombocytopenia was seen in 7% of cases and no patient had absolute Neutrophil count (ANC) < 800 cells/μL. [49]

Additionally In a prospective study done from 2002-2003 in one hundred HIV/AIDS infected previously HAART naïve adult Nigerians aged 18-58years ;anemia occurred in 80% of subjects while Leucopenia, Neutropenia and Thrombocytopenia were found in10%, 24% and 10% of the study population respectively.[50]

Similarly In cross-sectional study done in Benin City, Nigeria in 2009 to assess Prevalence of anemia using WHO criteria among 457 HIV-infected patients of whom 217 were on HAART and 240 HAART naïve, the overall mean age was 30.57 ±7.81 years; the overall prevalence of anemia was 60.6%. [51]

Whereas In 1999 a cross-sectional study conducted in Zimbabwe to describe the hematologic features of HIV infection ;cytopoenia was found in 47.5% of the HIV infected patients and the most frequent abnormalities were lymphopoenia, anemia, neutropenia, thrombocytopenia,

eosinophilia and leucopenia in 31.5%; 30.8%; 29.6%; 24.7%; 23.5% and11.7% of cases respectively.[52]

While in a cross-sectional study conducted at Lagos State University Teaching Hospital (LASUTH) in 2010 in a total of 205 treatment-naïve HIV-infected patients whose mean age was 35.5years ±9.16 years ;to determine prevalence of cytopoenia and it's relationship with degree of immune suppression ; cytopoenia was present in About one-fifth of the patients at enrollment, of which 24.2%, 26.8% and 16.1% had anemia (PCV<30%), leucopenia (white blood cell <4,000/µL) and thrombocytopenia (platelet count <150,000/µL) respectively.[53]

Moreover In a retrospective Cohort study done in South Korea from 2005 to 2010 in 472 HIV patients on ART whose median age was 35 years (27-44) ;to determine the Hematological impact of HIV itself; showed that anemia, neutropenia, thrombocytopenia, lymphopoenia, isolated cytopoenia, and Bicytopenia was seen in3.0% (14/472); 10.0% (47/472); 2.4% (12/472); 25.7% (121/470); 11.2% (53/472); and 2.1% (10/472) of cases respectively.[54]

B-Risk factors of cytopoenias in HIV/AIDS

In one retrospective cohort study done in nine United States cities from 1990-1996 to evaluate the 1-year incidence of anemia and the associated risk factors in 13,315 HIV-Infected Persons who were on ART: the incidence of anemia was 3.2%, 12.1% and 36.9% for persons with HIV infection but not AIDS (6,094), for persons with immunologic AIDS but not clinical AIDS (2,579), and for persons with clinical AIDS (4,642) respectively. And the Incidence of anemia was positively associated with clinical AIDS, immunologic AIDS, neutropenia, thrombocytopenia, bacterial septicemia, black race, female sex, prescription of zidovudine, fluconazole, and ganciclovir, and negatively associated with prescription of trimethoprim-sulfamethoxazole. [55]

Similarly the study conducted in Zimbabwe to describe the hematologic features of the HIV infection and compare the features in the different clinical stages of the disease: The Frequency of anemia was greater in the AIDS and symptomatic groups (43.4% and 24.5% respectively) than in the carriers (6.7%), while the frequency of other cytopoenias was about the same in all groups. [52]

Additionally a study done in Rwanda in 2010 to determine the prevalence and risk factors of anemia and influence of HAART on anemia among 200 HIV–infected women who were all greater than 18 years of age ,whose first line ART regimen consisted of D4T or AZT plus lamivudin plus neverapin or efaverenze: the prevalence of anemia was 29% and it was significantly different between the different HIV stages and was9%, 26%, 40%, and 88% for clinical HIV stage I, II, III, and IV respectively ($P < 0.001$). Other Risk factors identified were; lower body mass index (odds ratio [OR] = 3.4, 95% confidence interval [CI] = 2.4–4.1), zidovudine use (OR = 1.14, 95% CI = 1.01–1.29), CD4 lymphocyte count < 200 cells/μL (OR = 2.41, 95% CI = 2.01–3.07) and lack of HAART (OR = 1.44, 95% CI = 1.21–1.67). [56]

As to the study conducted at LASUTH to determine the prevalence of cytopoenia and its relationship to the degree of immunosupression:

The degree of cytopoenia was directly related to the degree of immunosupression: Anemia was found in 5.26%,18.75%,43.75%,50% and 71.42% of patients with CD4 count >500 cells/μL, 350–500 cells/ μL, 200–350 cells/ μL, 100–200 cells/ μL and <50 cells/ μL respectively P-value 0.000. Leucopenia was found in10.5%, 26.31%, 31.25% and 55% of patients with CD4 count >500 cells/μL, 350–500 cells/ μL, 200–350 cells/ μL, and 100–200 cells/ μL respectively P-value 0.06. Whereas thrombocytopenia was found in 13.15%, 15.7%, 12.5%, 25%, 53.3% and15.28% of patients with CD4 count >500 cells/μL, 350–500 cells/ μL, 200–350 cells/ μL, 100–200 cells/ μL, 50–100 cells/ μL and <50 cells/ μL respectively. [53]

C-Pattern of anemia in HIV/AIDS

Etiologies of anemia in HIV/AIDS are multifactorial, as to the cross sectional observational study conducted in eastern India to evaluate the etiologies underlying anemia in 46 randomly selected anemic AIDS patients: the RBC morphology was Normocytic Normochromic, Microcytic hypo chromic and Macrocytic in 63%,28% and 9% patients respectively. As to this study Anemia of chronic disease was the commonest etiology (37%) followed by HIV related myelodysplastic syndrome (31%) and iron deficiency anemia (13%). [48]

Whereas the perspective study done in HAART naïve adult Nigerians: anemia occurred in 80% of subjects while 20% were non-anemic and of the anemic patients Red cell morphology was

Normochromic Normocytic and hypochromia and anisopoikilocytosis in 64% and 36% of cases respectively.[50]

D-Impact of HAART in cytopoenias in HIV/AIDS

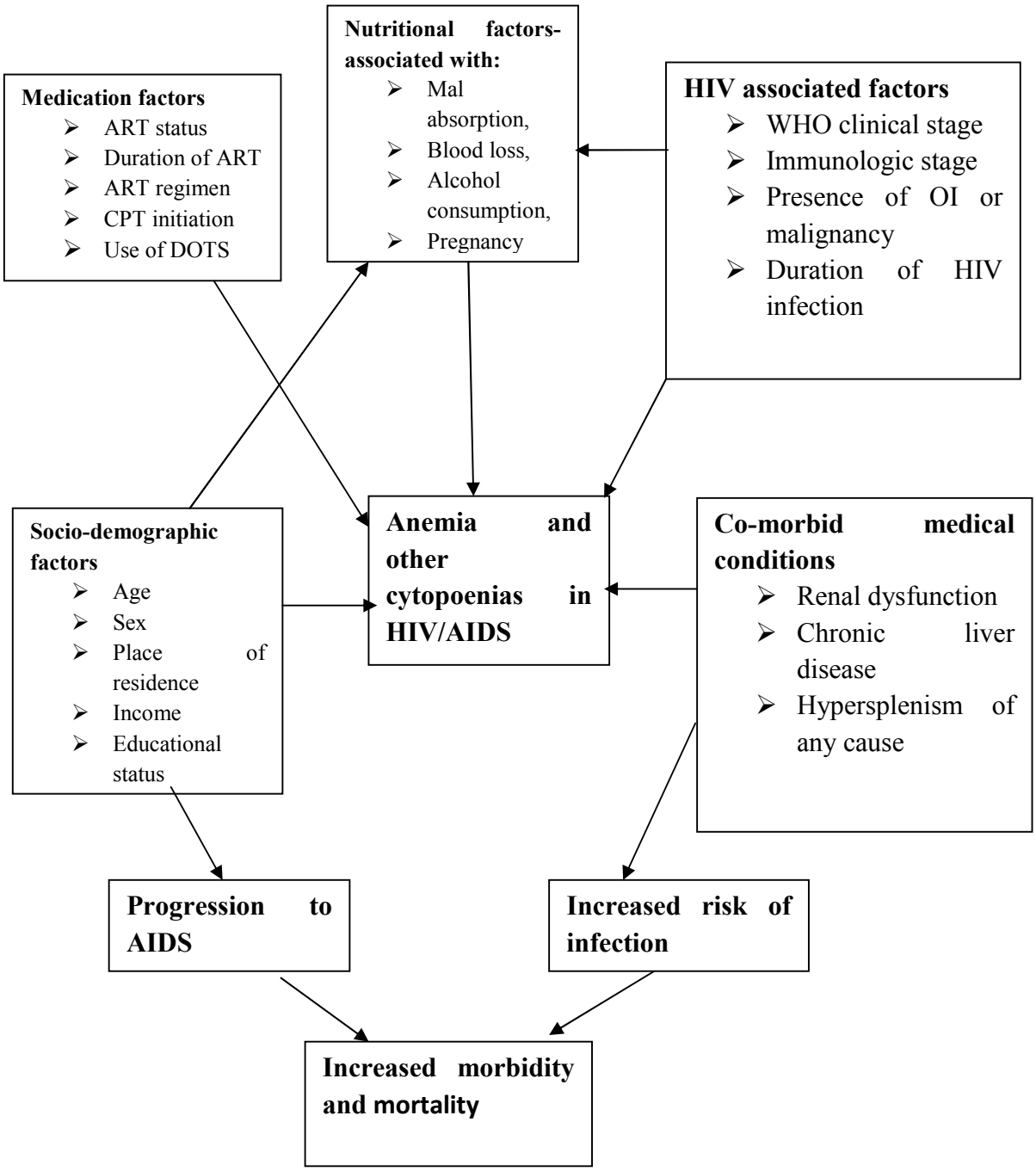
As to the cross-sectional study done in Benin City, Nigeria: the Prevalence of anemia was significantly higher among HAART naive patients (69.17%) than in those on HAART (51.15%) ($P < 0.001$) and Of HAART naive patients the prevalence was higher in males (76.42%) than in females (63.43%) ($P < 0.05$). [51]

In 2011 a Hospital based cross-sectional study conducted in Ghana to assess the impact of HAART in resolving hematological complications in a total of 442 PLWHA comprised of 276 HAART naive patients whose mean age was 33.42±0.88years and 166 patients who were on HAART(for a period of 3 months or more) whose mean age was 36.91±0.77years: The risk of developing anemia (Hb ≤ 10.5) was higher in HAART-naïve (63%) patients as compared to those on HAART (46%) ($X2 = 10.68$, $P = 0.0011$). Furthermore, HAART-naive patients had a higher risk (3 times at risk) of developing moderate to severe (Grade 2 and Grade 3) anemia compared to their counterparts on HAART (($P <= 0.0005$). The study also showed that there was a significant difference in the relative risk of developing lymphopoenia in HAART-naive patients as compared to those on HAART (16.7% and 5.3% respectively) ($X2 = 11.07$, $P = 0.0009$). However there was no significant difference in the relative risk of developing neutropenia and thrombocytopenia in the study populations ($P > 0.05$). [57]

In the retrospective Cohort study done in South Korea to determine the Hematological impact of HIV itself and the effect of HAART on cytopoenia: only the initial AIDS status was significantly correlated with cytopoenia ($P < 0.0001$, HR=8.520, 95% CI=5.166-14.052) and after six months of HAART, cytopoenia was reversed in the majority of patients (thrombocytopenia, 100%; neutropenia, 91.1%; and anemia, 84.6%). [54]

Finally the study done in Rwanda to determine the prevalence and risk factors of anemia among 200 HIV–infected women and the influence of HAART on anemia: Showed that the mean ± SD hemoglobin level of 10.9 ± 1.6 g/dL at HAART initiation significantly increased to 12.3 ± 1.5 g/dL in 8 months ($P < 0.001$).[56]

Conceptual framework:



Significance of the Study

Hematological abnormalities are among the most common complications of HIV both before and after HAART treatment and have been documented to be the second most common cause of morbidity and mortality in HIV patients.

There is no study done in our country to determine the prevalence of hematologic abnormalities and risk factors in adult HIV-infected patients; particularly in JUSH. So the study will fill this gap and it can also help physicians giving care for HIV infected adults to have a routine habit of investigating these patients for hematological abnormalities so that they will detect it early in the course of the disease and accordingly manage to improve the quality of life of HIV-infected patients.

Additionally this study will be a base for large scale and longitudinal studies to explore the problem in depth.

Chapter III-Objective

3.1-General Objective

To investigate the hematological manifestations of HIV infection and the risk factors for cytopoenias among adult HIV- infected patients who were on HAART and HAART naive, in Jimma university specialized hospital at ART follow up clinic from July to August 2012

3.2- Specific objectives

1.To determine the prevalence of anemia, thrombocytopenia, leucopenia, neutropenia and lymphopoenia among patients on ART, not on ART and for all subjects

2.To describe the patterns of anemia among patients on ART, not on ART and for all subjects

3.To identify factors that are associated with anemia, thrombocytopenia, leucopenia, neutropenia and lymphopoenia

Chapter IV: Methods and materials

4.1- Study Area and period

This study was conducted in JUSH from July to August 2012 for one month. JUSH is found in Jimma city, which is located in south west of Ethiopia 350km from the capital Addis Ababa. It is one of teaching and referral hospital in the country. The hospital has a bed capacity of 450 and a total of more than 750 staffs of both supportive and professional and gives health service at inpatient and outpatient level as being referral centre for 15 million populations in south west region of the country.

As one of the outpatient services, the hospital has TB/HIV care center that provides voluntary counseling and testing, care and treatment for TB and HIV infected patients. The ART clinic is staffed with internists, pediatricians, residents, trained nurses, a pharmacy technician, a data clerk and supporting staffs and gives service for five days per week and around 30 patients get follow up care each day.

4.2- Study Design

A hospital based cross sectional study was conducted.

4.3- Populations

4.3.1- Source Population

At the time of the study there were a total of 5954 HIV-infected adult patients on follow-up care, of which 2149 were on ART and 3805 were on pre-ART care.

4.3.2- Study Population

Among HIV-infected adult patients who visited the ART clinic during the study period 130 HAART and 231 HAART naïve, total 361, patients were included in the study.

4.4 - Exclusion criteria

Patients with the following conditions were excluded from the study:

1-Patients receiving or had received chemotherapeutic agents for any malignancy within 6 months prior to the study period; as anti cancer drugs can cause bone marrow suppression or cytopoenias unrelated to HIV/AIDS and it's complications (1 patient).

2- Patients who had received blood transfusion within 3 months prior to the study period; because the reversal of cytopoenia by blood transfusion could mask the effects of HIV on the hematological status (3 patients).

3- Patients who refuse to become part of the study.

4.5-Sample size determination

Taking estimated prevalence of cytopoenias (anemia, neutropenia, lymphopoenia and thrombocytopenia) among adult HIV-infected patient [p] 50% and allowing an error of 5% of detecting the estimated prevalence of cytopoenias by chance alone [d]with 95% confidence interval, the sample size was calculated using one proportion formula:

$$n_o = \frac{[Z\ \alpha/2]^2\ p[1-p]}{d^2}$$

Where

n_o= calculated sample size

Z α/2 = 1.96

P= prevalence rate of cytopoenias among adult HIV-infected patient which was unknown (0.5).

d=0.05

Using the above formula the calculated sample size will be 384.

Since the sampling was from finite population, the calculated sample size was corrected using the correction formula:

$$n = \frac{n_o}{1 + (n_o / N)}$$

Where no =is calculated sample size

Where n=is the corrected sample size

N= total number of patients who had been on regular follow up (in registration book) = 5954

Using this formula the Corrected sample size was 361.

4.6-Sampling method and procedure

Since the prevalence and severity of hematologic abnormalities in HIV-infected individuals primarily depends on whether an individual is on ART , Quota sampling technique was used to select subjects by classifying the study population into: on ART(on HAART) and pre ART care (HAART naïve) groups.

The total numbers of HIV-infected adult patients on regular follow up: 5954

HIV-infected patients on ART (on HAART): 2149

HIV-infected patients on pre ART care (HAART naïve): 3805

To take a sample of 361 HIV-infected patients;

The sample size of subjects was allocated to each category proportionate to size:

On ART=2149 (361÷5954)= 130

On pre ART=3805 (361÷5954)= 231

Each client who came to the clinic during study period was evaluated for eligibility to be included in the study. Those who did not have any of the exclusion criteria were included in the study consecutively for either of the 2 groups. The selection and inclusion of patients was continued until the specific number of patients (quota) was obtained for each category.

4.7-Study variables

4.7.1-Dependent Variables

Primary outcomes were the prevalence of anemia, leucopenia**,** lymphopoenia, neutropenia and thrombocytopenia. The secondary outcomes were Pattern of Anemia.

4.7.2- Independent Variables

The independent variables were socio-demographic characteristics (like age, sex, residency--), clinical data (like WHO clinical stage of HIV, updated CD4 count, duration since HIV diagnosis, use of ART, ART regimen, duration of ART use------) and laboratory CBC result.

4.8--Methods of data collection

4.8.1- Data collection instrument, tool and procedures

Up on coming to the ART clinic for their regular follow up; patients were assessed for eligibility and asked for consent to participate in the study, for those who agreed and were eligible for the study clinical interview and review of medical records were done for socio-demographic characteristics, clinical and medication data.

Data were collected using **a** pre-tested a structured questionnaire. The questionnaire had three parts. The first section was socio-demographic characteristics of patients'. The second section was on clinical data including duration of HIV infection, WHO clinical stage, history of blood loss, transfusion history, presence of: opportunistic infection, malignancy and other co morbidity. The third part was on medication data including ART status, ART regimen, ART duration, use of CPS and CD4 count determined within prior 6 months. The questionnaires (data) of patients on ART were numbered (coded) separately from those on pre-ART care.

For laboratory measurements a 3ml of blood sample was taken from the patients at the clinic and was dispensed into vacutainer EDTA tube, mixed and labeled. Then after the samples were transported to the hospital laboratory and in the laboratory each sampled blood was analyzed within two hours using DYW 1800 ABBOH auto analyzer machine for CBC profiles. CD4 count was done only for patients who did not have an updated CD4 count (with in prior 6 months).

4.9-Data collectors

A total of 8 people namely one third year internal medicine resident, two nurses who were working at ART follow up clinic and had training certificate and experience in care and treatment of patients with HIV, three laboratory technicians, and one sample transporter were involved in the study for a total of 30 days. In addition one qualified laboratory technician supervised the data collection process and laboratory qualities. The principal investigator supervised the overall activities during the data collection period.

4.10-Data quality control

Pretesting of the questionnaire were done in 20 HIV-infected adult patients in another health institution (ALERT hospital) before data collection to ensure logical sequence and skip patterns of the questions. Necessary correction of questionnaire were made accordingly after the pre-test.

Data collectors were trained for one day on the objectives of the study, each variable on the questionnaire and its implication, demonstration and practical session on interviewing, record reviewing, laboratory sample collection, labeling, transportation, storage and testing procedure as per standard testing procedure. A manual of study procedures were provided to each data collector which included; a review of the general procedure, a detailed discussion of every data item to be collected and a discussion of how to resolve potential problems that may occur during data collection.

During the training, detailed precautions as to the quality of reagents, amount, expiry, timing, and duration for test procedure as well as the quality of laboratory instruments and materials got emphasis with the objective of getting quality data.

During data collection, the principal investigator ensured quality data collection by supervision, on spot corrective action and recollecting data on 5% of the study population.

Each day the principal investigator reviewed all collected data, he checked for completeness and internal consistency and took immediate remedial action accordingly.

The same code was used on patient's card, on questioner, on the sampled blood and laboratory CBC results and were analyzed as to their respective code.

Every patient was registered only once in the study. To ensure this, the charts of the patients included in the study were marked with a sign.

4.11-Data processing, analysis and interpretation

The collected data were categorized, coded, entered onto a computer, cleaned (verified) and analyzed using SPSS for Windows version 20 by the principal investigator. Descriptive analysis was done to determine the prevalence of anemia, leucopenia, neutropenia, lymphopoenia and thrombocytopenia; and patterns of anemia; and were presented using tables, diagrams and summary measures as appropriate. Before analysis continuous variables were checked for whether normally distributed or not using normal graph curves. Normally distributed Continuous variables were compared with dependent variables using T- test and ANOVA; when ANOVA revealed significant difference further post hoc-multivariate comparison were done. Categorical

variables like sex, WHO clinical HIV stage**,** ART status, ART regimen, use of anti tuberculosis therapy, use of co-trimoxazole prophylaxis therapy, pregnancy status, presence of opportunistic infection, presence of co-morbidity and others were compared with dependent variables using chi- square test and fisher exact test when appropriate (when the expected number of frequencies per cell In a 2x2 table was less than 5). And when association was found the odds ratio was used to measure the strength of association. Finally for variables which had statistically significant association with dependent variable multiple logistic regression analysis were done to come up with the independent predictors of outcome variables (dependent variable). All tests were two tailed and statistical significance was considered at $p<0.05$ with 95%CI.

4.12-Ethical considerations

Before starting data collection ethical clearance was obtained from ethical review committee of Jimma University, college of public health and medical sciences and permission to conduct the study was obtained from JUSH. Purpose and objectives of the study was explained to respective head in the department and the clinical director. After informing patients about the objective of the study and the confidentiality of the data, verbal informed consent was obtained from study subjects before inclusion into the study. To ensure confidentiality of data, study subjects was identified using codes and unauthorized persons had no access to the collected data, information obtained from interview, chart review and laboratory results were used only for the purpose of this study and blood was drawn using standard universal precaustions of sample collection from clients. Furthermore, the CBC findings were attached to the patient card and were utilized for proper management of the patients.

4.13-Dissemination of the results

The result of the study was submitted to college of medical and public health, the department of internal medicine and JUSH. Moreover the research findings can be presented on workshops, seminars, and on other professional gatherings. The paper can also be published in a peer reviewed journals.

4.14 – Operational definitions

Anemia, leucopenia, neutropenia, lymphopoenia, thrombocytopenia and Patterns of anemia were defined based on World Health Organization (WHO) definition of hematologic abnormalities. (45, 46, 47, 48, 49, 50)

Accordingly, anemia was defined as follows:

Hb concentration less than 13gm/dl for adult male

Hb concentration less than 12gm/dl for adult female and

Hb concentration less than 11gm/dl for pregnant women

Severity of anemia was also classified based on WHO grading of anemia as follows :

Grade 1 (Mild Anemia): 10 g/dl - cutoff point for ages

Female-10.0–11.9 g/dl

Males-10.0–12.9 g/dl

Pregnant women-10.0-10.9 g/dl

Grade 2 (Moderate Anemia): 7-9.9 g/dl

Grade 3 (Severe Anemia): below 7 g/dl

Patterns of anemia were classified as follows:

Normocytic -MCV 80 -100fL

Microcytic- MCV <80fL

Macrocytic - MCV >100fL

Normochromic- MCH 27 -32 pg

Hypo chromic- MCH <27 pg

Leucopenia was defined as WBC count <4000 cells/μL.

Absolute Neutrophil count and total lymphocyte count were calculated as:

ANC= WBC× ((Segments/100) + (Bands/100))

TLC= WBC× (Lymphocyte differential/100)

Neutropenia was defined as absolute Neutrophil count (ANC) of less than 1000 / μL.

Lymphopoenia was defined as total lymphocyte count <800 cells/μL.

Thrombocytopenia was defined as platelet count < 150 × 103/μL.

Isolated cytopoenia was defined as presence of anemia, thrombocytopenia, or neutropenia

Bicytopenia was defined as presence of any 2 of the following 3 anemia, thrombocytopenia and neutropenia

Pancytopenia was defined as presence of anemia, thrombocytopenia, and neutropenia all together

Immunologic classification

The study populations were classified in to three categories based on CD4 counts using the Center for Disease Control (CDC) criteria (51):

Stage 1 -CD4 count >500 cells /mm3

Stage 2 -CD4 count between 200 and 499 cells /mm3 and

Stage 3 CD4 count <200 cells /mm3

Immunologic AIDS was defined as CD4 count of <200/μL, regardless of the presence of +symptoms or opportunistic diseases according to the Center for Disease Control (CDC) criteria. (51)

Clinical AIDS was defined as a self-reported history of a clinical AIDS-defining condition using the 1993 Centers for Disease Control (CDC) criteria. (51)

Clinical staging

Based on WHO clinical staging of HIV disease in adults and adolescents the study populations were classified in to stage 1, stage 2 , stage 3 and stage 4 (52).

HAART use was defined as receipt of two nucleoside reverse transcriptase inhibitors (NRTI) and one non-nucleoside reverse transcriptase inhibitor (NNRTI) or one protease inhibitor (PI).

Chapter V: Results

5.1-Socio-demographic characteristics

361 HIV infected individuals were involved in this study, made up of 149(41.3%) males and 212(58.7%) females. The overall mean age for the study population was 34.7±10.1 years. Majority of the study subjects (87.8%) were living in urban areas and more than three fourth (77.5%, n=280) of them had at least primary school education or more. Nearly half (49.6%, n=179) of the participants were married , 18.8% single , 21.6% divorced and 10% were widows or widowers. 22.7% of the individuals were dependent and 54% earned monthly personal income below 500 birr (Table 1).

Table 1: Distribution of socio-demographic characteristics of the study subjects, at JUSH from July-August 2012

Variables	On ART N (%)	Not on ART N (%)	Total N (%)
Age in years			
16-26	17(13.1)	61(26.4)	78(21.6)
27-37	56(43.1)	107(46.3)	163(45.2)
38-48	40(30.8)	46(19.9)	86(23.8)
49-59	15(11.5)	7(3.0)	22(6.1)
60-70	2(1.5)	10(4.3)	12(3.3)
Total	130(100)	231(100)	361(100)
Mean age	33.5±10.3	36.8±9.5	34.7±10.1
Sex			
Male	50(38.5)	99(42.9)	149(41.3)
Female	80(61.5)	132(57.1)	212(58.7)
Total	130(100)	231(100)	361(1000)
Residence			
Urban	113(86.9)	204(88.3)	317(87.8)
Rural	17(13.1)	27(11.7)	44(12.2)
Religion			
Muslim	42(32.3)	90(39)	132(36.6)
Orthodox	72(85.4)	118(51.1)	190(52.6)
Protestant	15(11.5)	19(8.2)	34(9.4)

Catholic	0(0.0)	3(1.3)	3(0.8)
Other	1(0.8)	1(0.4)	2(0.6)
Ethnicity			
Oromo	62(47.7)	118(51.1)	180(49.9)
Amhara	28(21.5)	48(20.5)	76(21.1)
Keficho	16(12.3)	22(9.8)	38(10.5)
Gurage	5(3.8)	11(4.8)	16(4.4)
Dauro	12(9.2)	18(7.8)	30(8.3)
Other	7(5.4)	14(6.1)	21(5.8)
Marital status			
Married	57(43.8)	122(52.8%)	179(49.6)
Single	17(13.1)	51(22.1%)	68(18.8)
Divorced	34(26.2)	44(19.0%)	78(21.6)
Widowed	22(16.9)	14(6.1%)	36(10.0)
Educational status			
Illiterate	24(18.5)	44(19.0)	68(18.8)
Read and write	5(3.8)	8(3.5)	13(3.6)
Primary school	49(37.7)	103(44.6)	152(42.1)
Secondary school	37(28.5)	67(29.0)	104(28.8)
Diploma level	12(9.2)	9(3.9)	21(5.8)
Degree and above	3(2.3)	0(0.0)	3(0.8)
Occupational status			
Farmer	2(1.5)	4(1.7)	6(1.7)
Merchant	8(6.2)	21(9.1)	29(8.0)
Go-employee	30(23.1)	27(11.7)	57(15.8)
Private work	37(28.5)	59(25.5)	96(26.6)
Daily laborer	24(18.5)	67(29.0)	91(25.2)
Dependent/retired	29(22.3)	53(22.9)	82(22.7)
Monthly personal income			
<500	84(64.6)	111(48.1)	195(54.0)
500-1000	21(16.2)	91(39.4)	112(31.0)
1001-1500	13(10.0)	24(10.4)	37(10.2)
>1500	12(9.2%)	5(2.2%)	17(4.7%)

5.2-Base line clinical profile

According to WHO clinical staging, about two third (64% , n=231) of the study subjects were in stage I and II ,while 36% (n=130) of the individuals had advanced clinical stage (stage III and IV). 13.3% (n=48) of the individuals had active of opportunistic infection (OI) at time of the study, the commonest being tuberculosis (TB) seen in 54.2% (n=26) of cases, followed by chronic gastroenteritis, pneumonia and candidiasis (oral± esophageal) seen in 25% (n=12), 10.4% (n=5) and 10.4% (n=5) of cases respectively (Table 2).

Table 2: Clinical and immunological characteristics of the study subjects, at JUSH from July-August 2012

Variables	Females	Males	Total
	N (%)	N (%)	N (%)
WHO clinical stage			
Stage I	56(62.2)	34(37.8)	90(24.9)
Stage II	80(56.7)	61(43.3)	141(39.1)
Stage III	66(64.7)	36(35.3)	102(28.3)
Stage IV	10(35.7)	18(64.3)	28(7.8)
Opportunistic infections			
TB	12(46.2)	14(53.8)	26(7.2)
Chronic GE	6(50)	6(50)	12(3.3)
Pneumonia	1(20)	4(80)	5(1.4)
Candidiasis	4(80)	1(20)	5(1.4)
No	189(60.4)	124(39.6)	313(86.7)
Total	212(58.7)	149(41.3)	361(100)
Co- morbidities			
HTN	2(100)	0(0)	2(0.5)
DM	0(0)	2(100)	2(0.5)
CLD	2(100)	0(0)	2(0.5)
No	208(58.6)	147(41.4)	355(98.3)
Total	212(58.7)	149(41.3)	361(100)

ART regimen			
TDF based1st	27(51.9)	25(48.1)	52(40)
AZT based1st	36(70.6)	15(29.4)	51(39.2)
D4T based1st	16(61.5)	10(38.5)	26(20)
TDF based 2nd	1(100)	0(0)	1(0.8)
Total	80(61.5)	50(38.5)	130(100)
Immunologic stage			
CD4 ≥500	53(69.7)	23(30.3)	76(21.1)
CD4 200-499	127(57.7)	93(42.3)	220(60.9)
CD4 <200	32(49.2)	33(50.8)	65(18.0)

Mean CD4 lymphocyte count for the study population was 380±221 (range 5-1421). The mean CD4 count of females and males were 406±232.6 and 343±199 cells/µl respectively. According to WHO classification of CD4 count, percent of patients with CD4 count>500,200-499 and <200 were 21.1% , 60.9% and 18% respectively,(Table 2 and 3).

Table 3: Age, clinical and hematological parameter distribution by gender of the study subjects, at JUSH from July- August 2012

Variables	Female	Male	Total
	Mean(range)	Mean(range)	Mean(range)
Age in years	32.1(16-70)	38.4(17-68)	34.7(16-70)
Duration of illness(in months)	31(1-117)	27.6(1-96)	30(1-117)
Duration of ART	41(1-96)	36(1-76)	39(1-96)
CD4 count	406(5-1377)	343(36-1421)	380(5-1421)
Hemoglobin(mg|dl)	11.9(4.3-15.6)	12.6(7.8-18.6)	12.2(4.3-18.6)
WBC x 10^3	6.01(1.8-13)	6.07(2.3-11.6)	6.03(1.8-13)
ANC x 10^3	3.55(1.1-9.9)	3.53(.92-8.4)	3.54(0.92-9.9)
TLC x 10^3	1.85(.35-5.1)	1.86(.54-4.3)	1.85(0.35-5.1)
Platelet count x 10^3	296.8(62-987	276.9(13-890)	288.59(13-987)

5.3- ART status of the study subjects

Of the 361 individuals, about two third (64% , n=231) were HAART naive and 36% (n=130) were taking HAART. 37.7%(n=80) of female and 33.6%(n=50) of male study subjects were on ART (see Table 2). Mean age of 33.5±10.3 and 36.8±9.5 years was obtained for HAART naïve and HAART patients respectively(p=0.02). The mean CD4 count of HAART patients (429±237) was 1.2 times higher than that of their HAART naïve counterparts (352±207) (p=0.002). Of HAART patients, over one third (40% , n=52) were taking tenofovir (TDF) based first line regimen, while 39.2% (n=51) and 20% (n=26) of individuals were taking zidovudine (AZT) and stavudine(D4T) based first line regimen, respectively. However, only one patient(0.8%) was taking (TDF based) second line regimen. The overall mean duration since HAART initiation was 39±24.5 months (range 1-96 months), it was 41±25.8 and 36±21.9 months for females and males respectively (Table 2, 3 4).

Table 4: Age, clinical and hematological parameter distribution by ART status of the study subjects, at JUSH from July-August 2012

Variables	Total	On ART	ART naive	p-value
	Mean (range)	Mean (range)	Mean (range)	
Age in years	34.7(16-70)	36.9(20-67)	33.5(16-70)	0.02
Duration of illness(in months)	30(1-117)	50.6(1-177)	18.9(1-90)	0.00
CD4 count	380(5-1421)	429(5-1314)	352(21-1421)	0.002
Hemoglobin(mg|dl)	12.2(4.3-18.6)	12.6(4.3-18.6)	12(7.6-16.9)	0.002
WBC x 10^3	6.03(1.8-13)	6.28(1.8-12.5)	5.9(2.4-13)	0.068
ANC x 10^3	3.54(0.92-9.9)	3.76(0.92-9.5)	3.42(1.2-9.9)	0.054
TLC x 10^3	1.85(0.35-5.1)	1.9(0.57-4.3)	1.8(0.35-5.05)	0.186
Platelet count x 10^3	288.6(13-987)	290.3(73-750)	287.6(13-987)	0.847

5.4- CBC profile of the study subjects

The mean(±SD) hemoglobin concentration for the study population was 12.2±1.86 (range 4.3-18.6).Lower hemoglobin concentration was seen in patients with female sex (11.9±1.65 g/dl for females vs. 12.6±1.65 g/dl for male, p<0.0001) and in HAART naïve patients (12±1.83 for HAART naive vs. 12.6±1.84 for patients on ART ,p=0.002) (see Table 4).

The mean WBC count for the study population was 6.03±1.86x 10^3 (range 1.8-13), it was 6.28 x 10^3 and 5.9 x 10^3 for HAART and HAART naïve patients respectively, (p=0.068) (see Table 4).

The mean (±SD) platelet count for the study population was 288.6±141.6 x 10^3 (range 13-987). There was no association between mean platelet count and gender, WHO clinical stages, ART status and ART regimen.. However, when analysis was done among patients on ART, patients on AZT based regimen had a higher mean platelet count (303.7±119.2 vs. 281.7±106.2 x 10^3 for non AZT based HAART patients, p=0.273) (see Table 4).

5.5- Prevalence and variable associated with cytopoenia

Anemia was seen in 186 (51.5%) patients. The prevalence of anemia were inversely associated with CD4 count (p=0.000) and it was positively associated with WHO clinical stages (in 23.3%, 57.4%, 62.7% and 71.4% of patients with WHO clinical stage I, II, III and IV, respectively, p=0.000) (Table 5,6,7).

Table 5: Frequency distribution of cytopoenia in the study subjects, at JUSH from July-August 2012

Cytopoenia	Female (n=212) N(%)	Male (n=149) N(%)	Total (361) N(%)
Anemia	100(53.8)	86(57.7)	186(51.5)
Leucopenia	28(13.2)	19(12.8)	47(13)
Thrombocytopenia	18(8.5)	22(14.80	40(11.1)
Lymphopoenia	13(6.1)	5(3.4)	18(5)
Neutropenia	0(0)	1(0.7)	1(0.3)
Isolated cytopoenia	100(47.2)	83(55.7)	183(50.7)
Bicytopenia	9(4.2)	13(8.70	22(6.1)

Table 6: Association of immunologic stages with cytopoenias in the study subjects, at JUSH from July-August 2012

Immunologic stage	Total No (%)	Anemia No (%)	P-value	Leucopenia No (%)	P-value	Thrombocytopenia No (%)	P-value	Lymphopoenia No (%)	P-value
>500	77 (21.3)	22(28.6)	0.000	3(3.9)	0.000	5(6.5)	0.261	0(0)	0.000
350-500	101 (28)	33(32.7)		6(5.9)		10(9.9)		0(0)	
200-349	118 (32.7)	77(65.3)		16(13.6)		13(11)		5(4.20	
100-199	42 (11.6)	34(81)		14(33.3)		9(21.4)		5(11.9)	
50-99	14 (3.9)	12(85.7)		4(28.6)		2(14.3)		6(42.9)	
<50	9 (2.5)	8(88.9)		4(44.4)		1(11.1)		2(22.2)	
Total	361(100)	186(51.5)		47(13)		40(11.1)		18(5)	

Table 7: Association of clinical stage with cytopoenia in the study subjects, at JUSH from July-August 2012

Clinical stage	Total No(%)	Anemia No(%)	P-value	Leucopenia No(%)	P-value	Thrombocytopenia No(%)	P-value	Lymphopoenia No(%)	P-value
Stage I	90(24.9)	21(23.3)	0.000	9(10)	0.132	9(10)	0.982	2(2.2)	0.138
Stage II	141(39.1)	81(57.4)		15(10.6)		16(11.3)		5(3.5)	
Stage III	102(28.3)	64(62.7)		16(15.7)		12(11.8)		9(8.8)	
Stage IV	28(7.8)	20(71.4)		7(25)		3(10.7)		2(7.1)	
Total	361(100)	186(51.5)		47(13)		40(11.1)		18(5)	

Lower prevalence of anemia was found in HAART patients (43% n=56) as compared to their HAART naïve counterparts (56.3% n=130) (p=0.021). However, there was no association between the prevalence of anemia and ART regimen (AZT vs. non AZT based) (p=.0.58). Mild, moderate and severe anemia were seen in 79.6%(n=148), 19.9%(n=37) and 0.5%(n=1) of the subjects respectively (Table 8).

Table 8: Distribution of categories of hematologic values of the study subjects, at JUSH from July-August 2012

Variables	On HAART No (%)	HAART naïve No (%)	Total No (%)	P-value
Hemoglobin (g/dl)				
Non anemic	74(56.9)	101(43.7)	175(48.5)	0.021
Anemic	56(43.1)	130(56.3)	186(51.5)	
Total	130(100)	231(100)	361(100)	
Anemia severity				
Grade 1	50(89.3)	98(75.4)	148(79.6)	
Grade 2	5(8.9)	32(24.6)	37(19.9)	
Grade 3	1(1.8)	0(0.0)	1(0.5)	
Total	56(100)	130(100)	186(100)	
WBC (cells/µl)				
≥4000	114(87.7)	200(86.6)	314(87.0)	0.890
<4000	16(12.3)	31(13.4)	47(13.0)	
Total	130(100)	231(100)	361(100)	
ANC (cells/µl)				
≥1000	129(99.2)	231(100.0)	360(99.7)	
<1000	1(0.8)	0(0.0)	1(0.3)	
Total	130(100)	231(100)	361(100)	
TLC (cells/µl)				
≥800	126(96.9)	217(93.9)	343(95.0)	0.318
<800	4(3.1)	14(6.1)	18(5.0)	
Total	130(100)	231(100)	361(100)	
Platelet count (cells/µl)				
≥150 × 103	121(93.1)	200(86.6)	321(88.9)	0.087
<150 × 103	9(6.9)	31(13.4)	40(11.1)	
Total	130(100)	231(100)	360(100)	

The severity of anemia were inversely associated with CD4 count (p=0.001) and positively with WHO clinical stages (p=0.007). Similarly use of ART was protective from severity of

anemia (8.9% for ART vs. 24.6% for ART naïve ,p=0.017). However, there was no association between the severity of anemia and gender of the study subjects (data not shown).

The most common pattern of anemia was normocytic normochromic in 98 (52.7%) cases followed by macrocytic anemia in 60 (32.2%) cases, normocytic hypo chromic anemia in 17 (9.1%) cases and microcytic hypo chromic anemia in 11 (5.9%) cases (Figure 1).

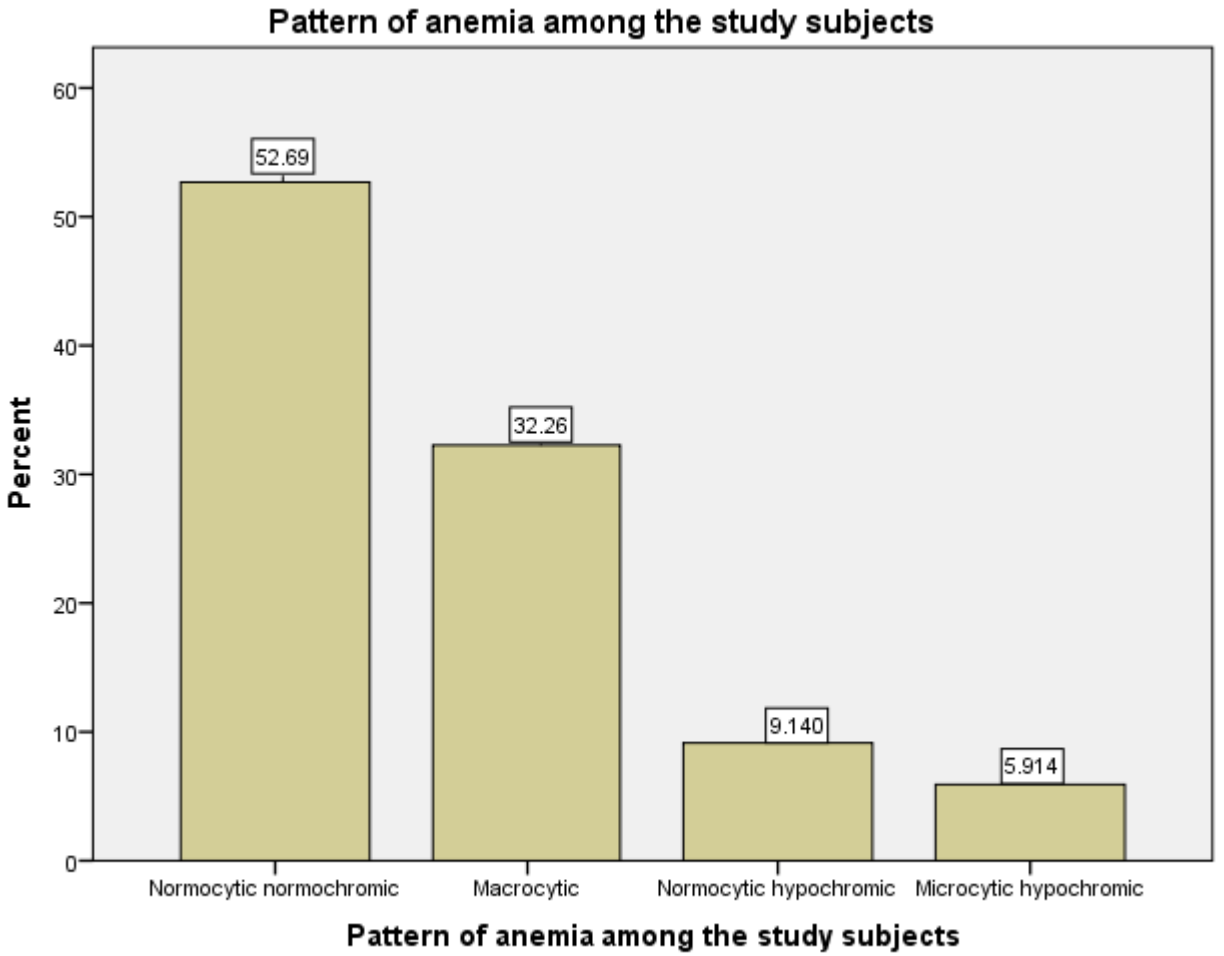


Figure 1: Bar-graph of pattern of anemia among the study subjects at JUSH, July-August 2012 (n=186)

Normocytic normochromic anemia was common in HAART naïve study subjects (60.8%) as compared to their HAART counterparts (33.9%), where as macrocytic anemia was more common in HAART patients (64.3%) as compared to HAART naïve subjects (18.5%) (p=0.000).

otherwise there was no difference between the patterns of anemia in relation to gender ,WHO clinical stage and CD4 groups (data not shown).

Leucopenia (WBC<4000/µL) was found in13%(n=47) of all participants. Leucopenia was common in patients with lower CD4 count(p=0.000), Whereas there was no association between the prevalence of leucopenia and WHO clinical stages , ART status and ART regimen (see table 5,6,7,8).

Thrombocytopenia (platelet<150,000/µL) was found in 11.1%(n=40) of the study subjects. Although statistically, not significant, among patients on ART, patients on AZT based regimen had a higher prevalence of thrombocytopenia (10.1%) as compared to patients on non-AZT based regimen (2%), (p=0.579). Otherwise there was no association between the prevalence of thrombocytopenia and immunologic stage, WHO clinical stages, ART status and gender of the study subjects (see table 5,6,7,8).

Lymphopoenia (TLC<800/µL) was found in 5%(n=18) of the participants. The prevalence of lymphopoenia had an inverse association with CD4 count ,p=0.000 . However, there was no association between the prevalence of lymphopoenia and WHO clinical stages ,ART status, ART regimens and gender of the study subjects (see table 5,6,7,8).

Neutropenia (ANC<1000/µL) was present in only one individual(0.3%) and isolated cytopoenia and bicytopenia were found in 50.7%(n=183) and 6.1%(n=22) of the participants respectively, and there was no patient with Pancytopenia (see table 5).

5.6-Risk factors for cytopoenias

A univariate analysis showed that many risk factors were associated with prevalent anemia. Risk factors associated with increased risk of anemia were: advanced WHO clinical stage OR 2.3(IC 95% 1.48-3.59; p=0.000), presence of opportunistic infection OR 10.2(IC 95% 3.94-26.5; p=0.000), lack of HAART OR 1.7(IC 95% 1.1-2.6; p=0.021), use of co-trimoxazole prophylaxis therapy OR 1.99(IC 95% 1.29-3.05; p=0.002), CD4 count <200 cells/µl OR 6.1(IC 95% 3.1-12.1; p=0.000), WBC count <4000/µl OR 2.78(IC 95% 1.41-5.48; p=0.004) and TLC <800 cells/µl OR 17.5(IC 95% 2.3-132.98; p=0.000). Presence of opportunistic infection, use of co-trimoxazole prophylaxis therapy and CD4 count <200 cells/µl were associated with an increased risk of anemia in the multivariate analysis with OR 5.72(IC 95% 2.05-15.97; p=0.001), OR 1.65(IC 95% 1.02-2.67; p=0.040) and OR 3.34(IC 95% 1.57-7.1; p=0.002) respectively (Table 9).

Table 9: Factors associated with anemia in the study subjects, at JUSH from July-August 2012

Variables	Anemia present n=186 N(%)	No anemia n=175 N(%)	COR (95% CI) p-value	AOR (95% CI) p-value
Clinical stage				
1Advanced(stage III and IV)	84(45.2)	46(26.3)	2.3(1.48-3.6) 0.000	1.59(0.93-2.72) 0.089
Early (stage I and II)	102(54.8)	129(73.7)		
Opportunistic infection				
1Present	43(23.1)	5(2.9)	10.2(3.94-26.5) 0.000	5.72(2.05-15.92) 0.001
Absent	143(76.9)	170(97.1)		
HAART status				
1HAART naïve	130(69.9)	101(57.7)	1.7(1.1-2.6) 0.021	1.53(0.91-2.56) 0.109
On HAART	56(30.1)	74(42.3)		
Use of CPT				
1On CPT	128(68.8)	92(52.6)	1.99(1.29-3.05) 0.002	1.65(1.02-2.67) 0.04
Not on CPT	58(31.2)	83(47.4)		
CD4 count				
1<200	54(29)	11(6.3)	6.1(3.1-12.1) 0.000	3.34(1.57-7.1) 0.002
≥200	132(71)	164(93.7)		
WBC count				
1<4000	34(18.3)	13(7.4)	2.78(1.41-5.48) 0.004	1.44(0.66-3.14) 0.356
≥4000	152(81.7)	162(92.6)		
TLC				
1<800	17(9.1)	1(0.6)	17.5(2.3-132.98) 0.000	7.26(0.867-60.74)
≥800	169(90.9)	174(99.4)		

A univariate analysis showed that use of co-trimoxazole prophylaxis therapy, CD4 count <200cells /µl, presence of anemia and presence of lymphopoenia were associated with increased risk of leucopenia with OR 3.06(IC 95% 1.43-6.55; p=0.005), OR 5.54(IC 95% 2.87-10.7; p=0.000), OR 2.78(IC 95% 1.41-5.48; p=0.004) and OR 6.23(IC 95% 2.32-16.74; p=0.000) respectively. In the multivariate analysis only CD4 count <200 cells/µl and use of co-trimoxazole prophylaxis therapy were associated with increased risk of leucopenia with OR 3.34(IC 95% 1.59-7.02; p=0.001) and OR 2.34(IC 95% 1.05-5.19; p=0.036) respectively (Table 10).

Table 10: Factors associated with prevalent leucopenia in the study subjects, at JUSH from July-August 2012

Variables	Leucopenia present n=47 N(%)	No leucopenia n=314 N(%)	COR (95% CI) p-value	AOR (95% CI) p-value
Use of CPT				
1On CPT	38(80.9)	182(58)	3.06(1.43-6.55) 0.005	2.34(1.05-5.19) 0.036
Not on CPT	9(19.1)	132(42)		
CD4 count				
1<200	22(46.8)	43(13.7)	5.54(2.87-10.7) 0.000	3.34(1.59-7.02) 0.001
≥200	25(53.2)	271(86.3)		
Anemia status				
1Present	34(72.3)	152(48.4)	2.78(1.41-5.48) 0.004	1.6(0.76-3.34) 0.211
Absent	13(27.7)	162(51.6)		
TLC				
1<800	8(17)	10(3.2)	6.23(2.32-16.74	2.78(0.908-8.54) 0.073
≥800	39(83)	304(96.8)		

A univariate analysis showed that there was no risk factor associated with prevalence of thrombocytopenia. It was found in 6.5%, 9.9%, 11%, 21.4%, 14.3% and 11.1% of patients with CD4 count > 500 cells/µl, 350-500 cells/µl, 200-349 cells/µl, 100-199 cells/µl, 50-99 cells/µl and < 50 cells/µl respectively (p=0.261). Although statistically not significant HAART naïve patients were more than 2 times at risk of having thrombocytopenia compared to those on HAART (p=0.087). Patients on non AZT based HAART were more than 5 times at risk of having thrombocytopenia compared to those on AZT based HAART , however it was not statistically significant (p=0.151) (see table 6,8).

A univariate analysis showed that many risk factors were associated with prevalence of lymphopoenia. Risk factors associated with increased risk of lymphopoenia were: duration since HIV diagnosis of ≤ 6 months OR 12.3(IC 95% 3.9-38.4; p=0.000), duration since HAART initiation of ≤ 6 months OR 26.07(IC 95% 2.52-269.3; p=0.006), CD4 count < 200 cells/µl OR 14.5(IC 95% 4.97-42.53), presence of anemia OR 17.5(IC 95% 2.3-132.98; p=0.000), presence of leucopenia OR 6.23(IC 95% 2.32-16.74; p=0.000) and presence of opportunistic infection OR

3.58(IC 95% 1.27-10.05; p=0.021). however in the multivariate analysis none of them were associated with increased risk of lymphopoenia (data not shown).

Chapter VI: Discussion

The prevalence of anemia in our patients, defined as a hemoglobin concentration of less than 13g/dl for men, 12g/dl for women and 11g/dl for pregnant women, was 51.5%, a value that aggress with the Benin city and India studies (49,51). We did not observe any difference in the prevalence of anemia between women (47.2%) and men (57.7%) (p=0.062). This result is in contrast with that found by others, who have observed a higher prevalence of anemia in women (55), who are more liable to iron deficiency. Also, we did not observe any difference in the prevalence of microcytic anemia between women (6%) and men (5.8%) (p=0.918).

This study confirms that anemia is directly related with the degree of immunosupression, a finding that aggress with those found by others (53,55,56): the lower the CD4 count the higher the prevalence of anemia.

We also proved that anemia is associated with advanced WHO clinical stage, a finding that is in accordance with the literature (52, 55, 56): the advanced the clinical stage the higher the prevalence of anemia.

In accordance with the Benin city and Rwanda studies (51,56), we did find a higher prevalence of anemia in HAART naïve patients compared to patients on HAART. We did not observe any relationship between hemoglobin level and AZT based HAART and non AZT based HAART, a finding that differs from that observed by others (55). The possible explanation, as showed by other studies, could be that the use of AZT is not associated with anemia when it is part of a HAART regimen (66).

Our study revealed that use of co-trimoxazole prophylaxis therapy is positively associated with prevalence of anemia, a finding in sharp contrast with that found by others, who have found a negative association between use of CPT and prevalence of anemia (55). One possible explanation could be that trimethoprim is a weak inhibitor of dihydrofolate reductase and in high doses, it has been implicated in Megaloblastic Pancytopenia, particularly in patients who are not on folate supplementation like our patients (67). Although the study found normocytic normochromic anemia as the most common pattern of anemia (52.7%), a finding that is in agreement with the literature (48,50), in contrast to them it also found a higher prevalence of Macrocytic RBC morphology (32.2%) which in part can explain the role of co-trimoxazole as an etiologic agent for the anemia.

Moreover this study found that, presence of opportunistic infection at time of the study was associated with an increased prevalence of anemia, irrespective of clinical stage and degree of immunosupression, which could perhaps be explained by anemia related to secondary infections.

The prevalence of leucopenia in our patients, defined as WBC count less than 4000/µl, was 13%, a value somewhat lower to those found by other studies ,which were done on HAART nave patients (48,53). The possible explanation could be the presence of HAART patients in our study which to a certain degree lowers the prevalence.

The study confirmed that leucopenia is directly related with the degree of immunosupression, a finding that agrees with the literature (53). Our study also revealed that use of co-trimoxazole prophylaxis therapy is positively associated with prevalence of leucopenia, a finding not addressed by others. The possible explanation , as mentioned above, could be Megaloblastic anemia which can occur as a side effect of co trimoxazole in the absence of folate supplementation.

The prevalence of thrombocytopenia (platelet count less than 150× 10) in our patients was 11.1%, a value somewhat similar to that found by others (49). This study confirmed that prevalence of thrombocytopenia is not associated with neither the degree of immunosupression nor with the clinical stage of HIV, a finding that is in accordance with the literature (52,53). Of most important this study also showed a higher prevalence of thrombocytopenia among patients on non AZT based HAART regimen as compared to those on AZT based HAART regimen. The possible explanation could be that AZT can rapidly increases platelet count in patients with HIV related thrombocytopenia (36,37).This effect is attributed to the decreased virus levels in the blood, and the decreased amount of anti-platelet antibodies while on HAART.

The prevalence of lymphopoenia in our patients, defined as TLC less than 800 cells/µl, was 5%, a value lower than found by a study undertaken on HAART patients (54). The possible explanation could be the presence of HAART patients in our study which to a certain degree lowers the prevalence. As expected, we found that patients with CD4 count less than 200cells/µl presented with a significantly increased prevalence of lymphopoenia. Our study revealed that lymphopoenia is inversely related with duration since HIV infection, higher prevalence observed in group of patients diagnosed in prior 6 months. The possible explanation could be that patients with HIV diagnosis of less than 6 months duration are unusual to be started on ART for so many reasons and as a result there will be ongoing T-lymphocyte damage. The study also revealed that

lymphopoenia is inversely related with duration of HAART treatment, higher prevalence observed in group of patients with HAART duration less than 6 months. The possible reason could be that hematologic recovery will occur after 6 months of HAART initiation (54).

Neutropenia, defined as ANC less than 1000/µl, was found only in one individual, a finding that agrees with the Indian study which found no patient with neutropenia (49). The mean Neutrophil count was lower in HAART naïve and immunologic AIDS patients, a finding that is in accordance with the literature (35,36,37).

Strength of the study:

-Adequate sample size- which can represent the HIV population in the study area

Limitations of the study:

-As the sampling technique used was non probability sampling, the sample may not be representative of all cases.

-Due to financial reason CD4 count was determined only for patients who had no an updated CD4 count, in the rest of patients CD4 count determined with in prior 6 months was taken from charts ,which might not represent the immunological status (CD4 count) of the patients by the time of the study.

Chapter VII: Conclusion and recommendation

7.1: Conclusion

In conclusion in this study anemia, leucopenia, thrombocytopenia and lymphopoenia were common among the study subjects in both HAART naïve and HAART patients. Of the cytopoenias, anemia was the most common manifestation and the most frequent patterns were normocytic normochromic and macrocytic forms, whereas neutropenia was rare.

Prevalence of anemia, leucopenia and lymphopoenia correlates with disease progression. Thrombocytopenia occurs independent of disease progression. The present study also showed that use of co-trimoxazole prophylaxis therapy was independently associated with an increased risk of anemia and leucopenia; a finding that needs to be strengthened by further study.

7.2: Recommendation

Based on these findings it is recommended that physicians giving care for HIV infected adults should routinely investigate and treat hematologic abnormalities in both pre and ART patients to reduce the morbidity and mortality of the patients.

Additionally, co trimoxazole prophylaxis therapy should be avoided in HIV-infected adult patients who have folic acid deficiency or who are pregnant, and instead the other alternatives for PCP prophylaxis can be used. And in the rest group of HIV patients co trimoxazole prophylaxis therapy should only be used if the potential benefit outweigh the possible hematologic risk (patients with CD4 count<200 cells/µl, prior bout of PCP……).

However, large scale and longitudinal studies by giving emphasis on association of co trimoxazole prophylaxis therapy and cytopoenia are recommended to strengthen and explore the problem in depth.

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Annex I. ART regimens

Adult 1st line regimens

1a= D4T +3TC+NVP

1b= D4T +3TC+EFV

1c= AZT +3TC+NVP

1d= AZT +3TC+ EFV

Other first line= TDF or ABC based regimen

Adult 2nd line regimens

2a= ABC + ddi +LPV/R

2b= ABC + ddi + NFV

2c= TDF + ddi +LPV/R

2d= TDF + ddi + NFV

Other TDF based 2nd line regimens

AZT based 2nd line regimens

Annex II. The 1993 Centers for Disease Control (CDC) criteria of clinical AIDS-defining conditions

Category C: Conditions listed in the AIDS surveillance case definition.
Candidiasis of bronchi, trachea, or lungs
Candidiasis, esophageal
Cervical cancer, invasive
Coccidioidomycosis, disseminated or Extrapulmonary
Cryptococcosis, Extrapulmonary
Cryptosporidiosis, chronic intestinal (>1 month's duration)
Cytomegalovirus disease (other than liver, spleen, or nodes)
Cytomegalovirus retinitis (with loss of vision)
Encephalopathy, HIV-related
Herpes simplex: chronic ulcer(s) (>1 month's duration); or bronchitis, pneumonia, or esophagitis
Histoplasmosis, disseminated or Extrapulmonary
Isosporiasis, chronic intestinal (>1 month's duration)
Kaposi's sarcoma
Lymphoma, Burkitt's (or equivalent term)
Lymphoma, primary, of brain
Mycobacterium avium complex or *M. kansasii*, disseminated or extrapulmonary
Mycobacterium tuberculosis, any site (pulmonary[a] or extrapulmonary)
Mycobacterium, other species or unidentified species, disseminated or extrapulmonary
Pneumocystis jiroveci pneumonia
Pneumonia, recurrent[a]
Progressive multifocal leukoencephalopathy
Salmonella septicemia, recurrent
Toxoplasmosis of brain
Wasting syndrome due to HIV

Annex III. The WHO clinical staging of HIV disease in adults and adolescents (54):

Stage 1

-Asymptomatic

-Persistent generalized lymphadenopathy

Stage 2

-Moderate unexplained weight loss (under 10% of presumed or measured body weight)

-Recurrent respiratory tract infections (sinusitis, tonsillitis, otitis media, pharyngitis)

-Herpes zoster

-Angular cheilitis

-Recurrent oral ulcerations

-Papular pruritic eruptions

-Seborrhoeic dermatitis

-Fungal nail infections

Stage 3

-Unexplained severe weight loss (over 10% of presumed or measured body weight)

-Unexplained chronic diarrhea for longer than 1 month

-Unexplained persistent fever (intermittent or constant for longer than 1 month)

-Persistent oral candidiasis

-Oral hairy leukoplakia

-Pulmonary tuberculosis

-Severe bacterial infections (e.g. pneumonia, empyema, meningitis, pyomyositis, bone or joint infection, bacteraemia, severe pelvic inflammatory disease)

-Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis

-Unexplained anemia (below 8 g/dl), neutropenia (below 0.5 x 109/l) and/or chronic thrombocytopenia (below 50 x 109/l)

Stage 4

-HIV wasting syndrome

-Pneumocystis jiroveci pneumonia

-Recurrent severe bacterial pneumonia

-Chronic herpes simplex infection (orolabial, genital or anorectal of more than 1 month's duration or visceral at any site)

-Oesophageal candidiasis (or candidiasis of trachea, bronchi or lungs)

-Extrapulmonary tuberculosis

-Kaposi sarcoma

-Cytomegalovirus disease (retinitis or infection of other organs, excluding liver, spleen and lymph nodes)

-Central nervous system toxoplasmosis

-HIV encephalopathy

-Extrapulmonary cryptococcosis including meningitis

-Disseminated nontuberculous mycobacteria infection

-Progressive multifocal leukoencephalopathy

-Chronic cryptosporidiosis

-Chronic isosporiasis

-Disseminated mycosis (histoplasmosis, coccidiomycosis)

-Recurrent septicemia (including non typhoidal Salmonella)

-Lymphoma (cerebral or B cell non-Hodgkin)

-Invasive cervical carcinoma

-Atypical disseminated leishmaniasis

-Symptomatic HIV-associated nephropathy or HIV-associated cardiomyopathy

Annex IV. Standard operating procedure

For

Complete blood count (CBC)

DISCIPLINE HEMATOLOGY

Instrument Celldyn 1800

PREPARATION DATE JULY, 2011

Prepared BY

.ORHB: ICAP/PLCU

.JUSH Laboratory

.

Version: Hema 01:……

Intended use

It is used to evaluate the compositions and concentration of blood;including all information related to white blood cell count, Neutrophils, lymphocytes, Monocytes, eosinophils, basophiles, red blood cell count, hemoglobin concentration, haematocrit, MCV, MCH and MCHC, red cell distribution width, platelet count, mean platelet volume, platelet distribution width, and plateletcrit. The results provides information concerning; oxygen carrying capacity of hemoglobin, Vascular integrity and the presence of infection in the body

Method

Auto method CBC determination

Reagent and equipment

1-Reagent [cyanide free lyses reagent]

2-test tube lavender top EDTA tube

3-Micrppipte

4-Micropipette tips

5-Celldyn 1800

Specimen

Whole blood using EDTA anticoagulants.

Principle

The CELL-DYN 1800 determines the CBC results as follows:

A suspension of blood cells is passed through a small aperture simultaneously with an electric current. The individual blood cells passing through the aperture introduces an impedance change in the aperture determined by the size of the cell. The system counts the individual cells and provides the size distribution. The lytic reagents rapidly and simultaneously destroy the erythrocytes and convert hemoglobin to a stable cyanide-containing pigment whose absorbance is directly proportional to hemoglobin concentration of the sample.

Procedure

1： Label tubes with the clients name/I.D No

2: Explain the blood drawing procedure to the client and reassure him/her

3: Wear the rubber glove and make the patient a comfortable position

4: Prepare the vacutainer tube and needle

5: Tie the tourniquet around the arm of the patient just above the bend in the elbow. the tourniquet should be positioned 7.5 cm to 10cm above the puncture site.

6: Tell the patient to clench his/her fist

7: Using the tip of the index finger examine the phlebotomy site , feel the vein, and decide exactly where to place the puncture

8: Disinfect the phlebotomy site by swabbing the skin in small outward circles with alcohol swab or cotton wool soaked in isopropyl alcohol. Do not touch the prepared puncture site with your fingers after disinfecting the skin.

9: Insert the needle directly into the vein and withdraw peripheral blood of approximately 4 to 5 ml in EDTA vacutainer tube.

10: Tell the patient to open his/her clenched fist

11: Release the tourniquet

12: Withdraw the needle from the vein and cover the puncture site cotton swab and hold (or have the subject hold) pressure at the puncture site for 3 minutes or until adequate homeostasis is visible.

13: Properly discard the used materials in a safe container and tell the subject to do so if handled the cotton swabs to stop the bleeding.

Normal value

Normal values vary with

. Age

. Sex

.Altitude

Interpretation

Depends on the cell type in consideration

Factors Affecting the CBC result (limitations)

Hemolyzed and clotted samples interfere with hematology test results

Quality control

Run quality control sample daily and compare with manual methods.

Annex V: Data Collection tool

A standard structured questionnaire format containing patients; socio demographic characteristics [age, sex, residency, religion, marital status, monthly income, occupation, educational status, ethnicity, smoking status, alcohol consumption and pregnancy status]; clinical data [duration of HIV infection, WHO clinical stage, history of blood loss, transfusion history, presence of: opportunistic infection, malignancy and other co morbidity] ; medication data [ART status, ART regimen, ART duration, use of CPS]; and CD4 count determined within prior 6 months; were used.

For laboratory CBC test [hemoglobin, WBC count and differential, platelet count, MCH, MCV, MCHC ..] a 3ml of blood, EDTA tube, and DYW 1800 ABBOH auto analyzer machine were used.

Annex VI. QUESTIONNAIRE

College of public Health and Medical Sciences, Department of Internal Medicine, Questionnaire on Hematologic abnormalities among adult HIV-infected patients on follow up at ART clinic, JUSH 2012.

Informed consent Form

In English

Good morning/afternoon!

My name is Tamirat Edie

I am final year internal medicine resident in JUSH.

I am going to conduct a research to partial fulfill the requirement for the certificate of specialty in internal medicine. The objective of the research is to determine the magnitude and assess possible associated factors of hematological abnormalities among HIV-infected adult patients in JUSH at ART follow up clinic**.** There is no a study done in our country and particularly in JUSH to address this issue. Thus doing this study will fill this gap and additionally it will be a base for large scale and longitudinal studies to explore the problem in depth. There are many HIV patients on follow up at ART clinic who are illegible for my study. You are welcome at the study. Your participation is purely voluntary & you have the right not to be participant in the study without any compromise of the treatment/care you ought to get. We kindly ask you to take some time to answer my questions. The interview will take few minutes.

All the data obtained will be kept confidentially & used for stated objectives only. The information you provide me and result of the sample will help to determine the magnitude and assess factors contributing to the problem & to state recommendations to improve quality of care given to HIV patients. We will give you some time to think on your participation and make decision.

If you decide to participate in the study you put on your signature at the end.

Thank you.

Name of participant_____
Signature _____ Date_____
Name of PI_____
Signature_____ Date_____

In Amharic

ዶ/ር ታምራት ኢደኤ

እ ታ

በመመረቂያ ፅሀፌ ላይ በማደርገው ጥናት ላይ ተሣታፊ እንዲሆኑልኝ በትህትና እጠይቃለሁ፡፡ ከጥናቱ ውስጥም በፈለግሆት ጊዜ መውጣት የሚችሉ ሲሆን ይህ ደግሞ ለሚሰጦት አገልግሎት ምንም አይነት ተፅኖ የለውም በተጨማሪም በጥናቱ ላይ ለመካፈል ከተስማሙ ከስር ባለው ክፍት ቦታ ላይ ፊርማዎትን ያስቀምጣሉ፡፡

አመሰግናለሁ

ፊርማ _____

In Afaan Oromo

Yaada waligalte afaan oroomotin

Dr Tamrate Eda’e

Waraqaa ebaakootiif qooranoo gaggeesuuf irrati akka iirmaatan kabajaan isiin isiin gaafadha. Qoora gaggeefamuu yeeroo barbaadaniitii bahuu kan dandeesan ta’uu isaa isiin ibsa.Haata’uu malee qoorannon waan midhaa geesisuu kan hin qabnee ta’uu isaa isiin huubachiisna.Haaluuma kanaan yoo yaada kanaa ol dheeramee waliigatan taanaan malatookeesaniina akka ibsaa.

Mallattoo_____

Questionnaire I.D: …….

Patient code No……..

Part I-Socio demography characteristics

1. Age (in completed years) _____

2. Sex M_____F_____

3. Place of residence (mention) _____ _____

4-Ethnicity [encircle].

I. Oromo II. Amhara III. Gurage

IV. Keficho V. Dawro VI. Other (specify) ---------

5-Religion [encircle].

A. Muslim B. Orthodox C. Protestant

D. Catholic E. Other (specify) ---------

6-Marital status [encircle].

A. Single (never married) B. Married

C. Divorced D. Widowed

7- Educational status attained [encircle].

A. Illiterate B. Read and write C. Primary school

D. Secondary school E.Diploma level F.Degree level and above

8-Monthly personal income: …….. Ethiopian birr

9- Occupation [encircle].

A. Farmer B. Merchant C. Government employee D. Daily laborer

E. Private work F. Retired G. Dependent

H. Other (specify) ---------

10- Alcohol consumption, Yes----- B. no-----

If yes, encircle on the type of drink (usually drunken)

A. Beer B. Wine C. Local areke

D. Tella E. Teje F. Other (specify) ---------

Volume consumed per day (in ml) -------

How frequent per week------

Volume consumed per week (in ml) -------

No. years drunken----------

11. Cigarettes smoking

A. Never

B. Yes

1. No. per day ---------- 2. No. years smoked---------

C. Quitted

1. No 2. Yes, if quitted, how many months/years back? --------

12. For females A. Are you Pregnant? Yes ____ No _____

If yes Trimester _____Wks.

B. Are you in Post menopause? Yes __ No __

Part II- Information on clinical data

1-Duration since HIV diagnosis

Year's _____ month's _____days_____

2-WHO HIV clinical stage (please tick one)

--------Stage I

--------stage II

--------stage III

--------stage IV

3-Does the patient have any Opportunistic infection currently (diagnosed by physician and documented)?

Yes----- no--------

If yes, which OI or malignancy mark (X) please

Chronic GE----------

Pneumonia----------

PCP----------

TB----------

TB & Pneumonia----------

PCP & Pneumonia----------

Others, specify------

4-Does the patient have any malignancy currently (diagnosed by physician and documented)?

Yes----- no--------

If yes, which malignancy mark (X) please

Lymphoma---------

Kaposi's sarcoma---------

Cervical cancer----------

Others, specify------

5-Other co-morbidity (not OI, not malignancy) identified, please mention

1 _____

2 _____

3 _____

4 _____

6-Documented Hx of blood loss

A. yes B. no

If yes, Site of loss----------

When? ----------

7-Have you ever been transfused with whole blood or other blood products?

A. yes B. no

If yes, When (how many months/years back)? ----------

Which blood products? Please mark (X)

Whole blood----------Packed RBC----------Platelets---------

Part III-Medication data

1- Is the patient on ART? Yes------ No--------

If yes

I-Duration since ART--------- Years ---------- months

II- Current ART regimen

First- line

A.1a B.1b C.1c D.1d

E.TDF based first line regimen

F. ABC based first line regimen

Second- line

G. 2a H.2b I.2c J.2d

K. Other TDF based second line regimen

L. AZT based second line regimen

2- Current Use of anti tuberculosis therapy? Yes------ No-------

If yes, Duration since Anti TB--------- months ---------days

3- Co- trimoxazole prophylaxis initiation

A. Present B. Absent

4- Current Use of any other drugs, please mention

I. ----------

II. ----------

III. ----------

IV. ----------

Part IV-Immunohematological data

4.1- Immunologic profile

1-CD4 count

Updated CD4 count (determined in prior 6 months) ---------

4.2- CBC laboratory result

1- Hemoglobin------g/dl 2-MCV------fl

3-MCH---------pg 4-MCHC---------%

5-WBC count---------/µl 6-ANC----------/µl

7-TLC----------/µl 8-Platelet count----------/µl

9- RDW