ADDIS ABABA UNIVERSITY
COLLEGE OF HEALTH SCIENCES
DEPARTMENT OF MEDICAL LABORATORY SCIENCES

ASSESSMENT OF IMMUNO-HEMATOLOGICAL PARAMETERS AMONG ADULT HIV POSITIVE PATIENTS BEFORE AND AFTER INITIATION OF 1J HIGHLY ACTIVE ANTIRETROVIRAL TREATMENT REGIMEN AT ST. PETER SPECIALIZED HOSPITAL, ADDIS ABABA, ETHIOPIA.

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AIDS	Acquired immune deficiency syndrome
ART	Anti-retroviral therapy
ARV	Antiretroviral
CBC	Complete Blood Count
CD4	Luster of Differentiation
DTG	Dolutegravir
EDTA2	Ethylene Di Amine Tetra Acetic Acid
FACS	Fluorescence Activated Cell Sorting
HAART	Highly Active Antiretroviral Therapy
HIV	Human immunodeficiency virus infection
ITP	Immune Thrombocytopenic Purpura
Nef	Protein Negative factor
NNRTIs	Non-Nucleoside Reverse Transcriptase Inhibitors
RBC	Red Blood Cell
SID	Subject Identification Number
TDF	Tenofovir
WBC	White Blood Cell
WHO	World Health Organization

Abstract

Background: Immuno-Hematological abnormalities are common in human immunodeficiency virus (HIV) infected individuals before and after ARV treatment However, the Immuno-hematological outcome of ARV treatment initiated usage is not well investigated.

Objectives: To assess hematological and CD4+ parameters among Adult HIV positive patient before and after initiation of ARV TREATMENT treatment at St. Peter specialized Hospital, Addis Ababa, Ethiopia, 2021.

Methods: Cross-sectional study was conducted from May to July, 2021 at St. Peter Specialized Hospital among Adult HIV positive patients. A total of Adult 422 HIV patients on ARV treatment for at a minimum of 3 months were selected using convenient sampling methods. Socio-demographic as well as clinical data of the participants was obtained using pre-tested structured questionnaires and review of medical records. Hematological parameters such as CBC was obtained using Beckman Coulter (DxH800) automated Hematology Analyzer and CD4+ parameter such as CD4 count was determined using BD FACS presto. Statistical analysis of the data was done using SPSS version 21. Paired t-test was used to compare dependent variables before and after initiation of ARV treatment and Binary Logistic regression was used to determine predictors of detected Immuno-Hematological abnormalities. P-value < 0.05 was considered as statistically significant.

Results: Of Adult 422 HIV positive individuals, about 64.7% were females. The mean age of study participants were42.16 (SD=±10.4years). The mean WBC, LYM, RBC, NEU, PLT, Hb and CD4, significantly improved after 3 months of ARV treatment. While, MCH and RDW significantly decreased after 3 months of ARV treatment without showing a significant improvement in MCV, HCT, MCHC, MPV and PDW after ARV treatment. On the other hand, the most common Hematological abnormalities detected were anemia (12.1), followed by Neutropenia (6%) and Thrombocytopenia (4%). Anemia was associated with Female Sex (AOR7.8, 95% CI:1.9-32.2, p<0.005) and Clinical stage III/IV (AOR16,95% CI:10.63-66.46, P<0.01).

Conclusion: There was a significant change in certain CD4+ and hematological parameters after initiation of the ARV treatment. Further studies are required to fully comprehend the outcome of the ARV regimen on hematological and CD4+ parameters.

Key words: Immuno-hematological, ARV treatment, Adult HIV Patients

1. Introduction

1.1 Background

Since the emergence of the HIV, it continues to be one of the world's most serious public health issues with over 79 million people have been infected of the virus and about not less than 36 million have died at the end of 2020. Globally, 37.7million people were living with HIV at the end of 2020. An estimated 0.8% of adults aged 15–49 years worldwide are living with HIV, of which nearly 1 in every 25 adults (3.6%) living with HIV accounts African region which remains most severely affected (1). Of adults living with HIV, 74%of adults were accessing antiretroviral therapy as of June 2021(2). In Ethiopia, 690,000 people were living with HIV and the percentage of people living with the virus among adults (15–49 years) was 0.9 % in 2020(3).

Human immunodeficiency virus infection (HIV) is a spectrum of conditions associated with various abnormalities amongst Hematological abnormality is the most common observed (4). Anemia, leukopenia and thrombocytopenia are the commonest hematological abnormalities found during HIV infection. These abnormalities present opportunity for further complications to arise to be as one most common causes of early death in HIV patient individuals (5). Recent studies indicated HIV involves almost all the lineages of the blood cells and of which Anemia is the most common hematologic abnormality to occur during disease stages of HIV.However, leukopenia, Neutropenia,lymphopenia and thrombocytopenia have also been occurred(6–8). The reasons for these derangements remain complex and multifactorial. HIV infects multipotent hematopoietic progenitor cells and establish latent cellular reservoirs, disturbs the bone marrow microenvironment and also causes immune deregulation (9,10).

Anemia is the earliest and most common hematologic abnormality affecting 60- 90% of patients in the late stage disease with lower CD4 count (11,12).

Thrombocytopenia, signifying a low platelet count, is another frequent usualfeature with during HIV infection. Recent report indicates thrombocytopenia as the second most frequent haematological complication in nearly 3 to 40% in HIV infections (13).

Leukopenia is also a common occurrence, especially in patients with advanced clinical stage of the disease; amongst neutropenia is the most common occurring in 10-30%. Lymphopenia primarily involves CD4 T-helper cells and is considered the classic hallmark of the disease as it worsens with the progression of the disease (14,15).

On the other hand, the use of antiretroviral drugs (ARVs) could also contribute to persistent hematopoietic suppression and subsequent hematologic abnormalities (16). Treatments with

many ARV drugs (combination treatment) are associated with a number of serious hematologic abnormalities and adverse effects that may ultimately limit the benefits of these ARVs (17). Therefore, ARV drugs could positively or negatively have an impact on hematological profiles, based on the combination used.

Recently, Ethiopia has shifted to the use of Dolutegravir based ARV treatment nationally since June 2020. Hence, Safety and acceptability following Dolutegravir based ARV treatment roll out as the country is very important. Thus, policy makers and program planners need scientifically sound evidence to evaluate the progress of this strategy and acceptability.

Moreover, to know the simultaneous effects of HIV infection and the ARV treatment on immuno-hematologic alterations, the Immuno-hematological outcome HIV-infected adults who were receiving these antiretroviral therapy was evaluated.

1.2 Statement of problem

Immuno-Hematological abnormalities are frequent manifestation of HIV and remain strong independent predictors of early death in adult HIV infected individuals. Across developing countries, while their progression of HIV disease escalates to severe state, most of Adult HIV patients do not began any of ARV treatment type (18).

More ever, as hematologic derangements could be due to either ARV drug or viral infection itself may further accelerate the severity of disease, putting the patients at risk of early death. This overall, undermines the health status and economic development adults. This in turn further spread of infection. Hence, investigating the impact of these drugs on hematological profiles and convenient ARV treatment regimen for observed hematologic derangement is imperative (11).

Previous findings from Sub-Saharan Africa suggest that anemia and other hematological abnormalities in patients receiving ARV is more common than in industrialized countries (11,17,19). Unluckily, there are still challenges in developing countries as often recommended ARV drug not be available (20).

In Ethiopia, the impact of different ARV treatment regimen on hematological profiles amongst Adult HIV patients is still poorly documented. This study was aimed to assess the effect of ARV therapy on hematological and CD4+ parameters among adult HIV patients (5).

On the other hand, recently, Ethiopia has shifted to use a treatment regimen, like Dolutegravir(DTG) based ARV treatment initiation for HIV infected individuals, so that, the treatment outcome of these regimen type should be known. The data emanating from it is help health care givers to consider the pros and cons of the treatment as well as for more reliable interpretation of hematology laboratory findings. Despite few reports present on the hematological profiles among adult HIV patients on ARV treatment in Ethiopia (5,11,12,17,21,22), which are entirely done on Prior ARV treatment regimen, the impact of the recently used ARV treatment regimen on hematological profiles is not well investigated. This study was aimed to address these limitations by determining hematological and CD4+profiles of initiated ART regimen, among adult HIV infected patients under care in St. Peter Specialized Hospital, Addis Ababa, Ethiopia.

1.3. Significance of study

Hematological abnormalities among HIV patients could be caused by the virus or ARV drug related. It was again shown that HIV drug therapy has a significant role in inducing some of the haematological changes in question and drug choice assessment is very important for avoidance of these unwanted side effects. Thus, knowing the impact of this ARV treatment on hematological parameters help the physician to consider drug of ART of choice having hematologic minimal toxicity.

This ultimately will decrease risk of early death due to hematological and CD4+ complications and enhance patient safety and improve health quality among person living with HIV.

The finding from this will also contribute to the limited body of knowledge on a prevalence of Hematological abnormalities of HIV patients on ARV treatment. It can also be used as an evidence to plan health education, promotion and implementation. Moreover, this study can serve as a source material for further researches referring to immuno-hematological profiles of adult HIV patients on anti-retroviral treatment.

2. Literature review

2.1 Overview of Immuno-hematological changes in HIV infection

There are broad implications of HIV on normal haematological state with hematological derangements involving all lineages of blood cells (23). The alterations and their aetiology describe the various further complications that could result due to HIV infection.

Prost et al., revealed that HIV hinder the hematopoietic dysfunction by disturbing HSCs essentially through the viral accessory protein Negative factor (Nef), having a substantial role in the pathogenesis of HIV associated hematopoietic dysfunction (24).

Moreover, the etiology and HIV induced hematologic abnormalities during HIV infection vary depending on lineage, disease stage and Bone marrow functionality (25). causes for HIV induced anaemia are related to anti-erythrocyte antibodies during HIV infection, tumour necrosis factor (TNF) implicated for in inhibition of bone marrow erythropoiesis through in vitro experiments and cytokines(IL-1), opportunistic infections(OIS), Some drug therapy can cause bone marrow suppression (26).

2.2 Prevalence of Hematological abnormalities

Study done by Harris et al., in 2008, across the Europe revealed that the magnitude of anemia before and After ART initiation was 35 % and 26% with mild (21.4%), moderate (3.7%) and severe (0.4%). According to this study, the detected anemia was associated with Sex (higher females) and CD4 count (increased with decreasing CD4) (27). Other studies also indicated Anemia is associated with, Low CD4 count (<200), Advanced clinical stage (III/IV) of HIV (12,17,18,28).

Study conducted by Thulasi *et al.*, 2016 showed among the total of 120 HIV cases, 77% had anemia, 21% had leucopenia and 5% had thrombocytopenia. The magnitude and severity of anemia, leucopenia, thrombocytopenia and other parameters was found to be more in patients before ARV treatment. This study added, the magnitude and severity of most of hematological abnormalities were inversely proportional to the CD4 count in before ARV therapy unlike those on ART (29).

As per study done by author Kaur *et al., 2017*, 77 % patients were found to be anemic, with anemic population on treatment naïve group 89.58% and after 6-12 months of treatment65.38%. This indicates improvement in hemoglobin after ART (30). this study have also noticed significant increment in WBC compared to base line (4.07 ± 0.25 x10^3 /µl vs 4.76 ± 0.15 x10^3/µl after ARV treatment (30).

A study in Iran ,2016 indicated mean of WBC and RBC after 6 months ART initiation was decreased significantly compared to the before ART initiation (31).

A study done in Italy showed that there was an improvement in the neutrophils count after receiving ART (32). In consistent with this study conducted in India and Denmark had reported increase in mean Lymphocyte count after ARV initiation compared to before ART commencement (4,33,34).

A retrospective study conducted in Southern Korea among 472 participants showed that the prevalence of neutropenia and lymphopenia were 0.89% and 4.17% in ART experienced patients, respectively (35). Contrary to this finding study conducted in Ghana reported the prevalence of leukopenia showed an increasing pattern after initiation of ARV TREATMENT. This abnormality could have resulted from the use of a zidovudine-containing ARV TREATMENT regimen, which can cause leukopenia by suppression of bone marrow production and cytotoxicity of T-cells eventually decreasing the survival of T-cells. In this study, Leukopenia is decreased by more than 50% after ART initiation (36). A cohort of HIV-infected adults in northwest Ethiopia showed overall, anemia was found in 25%(95% CI: 20.23 - 29.8%), of whom 2.5% (n=2) had severe and 21.2% (n=17) had moderate anemia. Having CD4 count below 200 cells/µl (AOR= 2.4, 95% CI: 1.3-4.9) was independent predictor of anemia. This study added, Thrombocytopenia was noted in 6.3% (95% CI: 3.58-8.9%) of the study participants (37) Study conducted in Beijing Ditan Hospital, China in 2021 on Thrombocyte Abnormalities in HIV-Positive Patients Before and After the Initiation of ART showed the prevalence was 2.65% among ART-naïve patients. This study noted onset incidence of thrombocytopenia was observed and varied with time (38).

A study conducted in Uganda also reported that the prevalence of thrombocytopenia was 17.8% among ARV TREATMENT naive and was 13.0% for clients who were on ART for up to 6 months. The study found a significant association between thrombocytopenia and other cytopenias, CD4 counts, ARV TREATMENT regimen, and deteriorating HIV stage (39).

A Study done by Damtie et al., 2021 noted, the prevalence of anemia, leucopenia, neutropenia, lymphopenia and thrombocytopenia were 37.1%, 22.8%, 8.4%, 10.5% and 17.1% before initiation of ARV TREATMENT and 17.4%, 34.2%, 18.8%, 13.1% and 8.3% after initiation of ARV TREATMENT, respectively. According to this study, there was a significant improvement in WBC,Hb, MCV, MCH, MCHC,), PLT and CD4 counts and decrease in RBC and RDW HIV patients before and after initiation of ART ($P<0.05$)(40). In similar with this study, other studies also indicated improvements in WBC, RBC, MCV, MCH and MCHC and decreased RDW after ART initiation (18,31,41).

Contrary to this find other study reported Decreasing in WBC count significantly after ART initiation (42).

Cross-sectional study done in DBRH, North-East Ethiopia,2020, showed the magnitude of leucopenia, neutropenia, and lymphopenia were 20.9%, 7.0%, and 6.6% before initiation of ART and 15.4%, 1.1, and 4.4% after initiation of ART, respectively (42). This finding is similar with finding of Enawgaw et al., North East, Ethiopia which noted the prevalence The prevalence of leucopenia, neutropenia, and lymphopenia were 20.9%, 7.0%, and 6.6% before initiation of ART and 15.4%, 1.1, and 4.4% after initiation of ART, respectively (18).

Another study conducted among adult HIV infected patients indicated that the mean platelet count was improved (222.2±119.2 x103/µl vs 228.6±88.9 x103 /µl) after at least six months of receiving ARV TREATMENT (31). In similar with this, other studies also reported incremental improvement in PLT after ARV TREATMENT initiation (43–46).

A cross sectional study on Prevalence of thrombocytopenia before and after initiation of ART among HIV infected patients at black lion specialized Hospital revealed that the prevalence of thrombocytopenia was 25% before ARV therapy and 4.1 % after ARV TREATMENT (47). This study further indicated, thrombocytopenia, increases as CD4 decreases.

2.3 Immunologic outcomes among HIV patients after ARV treatment

Recently, it is known that the management of HIV in positive patients have been based on ARV treatment.ARV treatment induces a marked reduction of viral replication as well as increases the CD4+ cell count. However, the response of CD4 count to ARV treatment is variable among HIV patients depending on base line CD4 status, ARV treatment type (48). As per the finding in Australia; A small but significant number of patients do not achieve CD4 T-cell counts .500cells/ml despite years of suppressive cART. clinical factors associated with CD4 T-cell recovery following long-term cART., faster time to achieve a CD4 T-cell count .500cells/ml was associated with higher baseline CD4 T-cell counts (p,0.001), younger age (p = 0.019) and treatment initiation with a protease inhibitor (PI)-based regimen vs. non-nucleoside reverse transcriptase inhibitor, NNRTI; (p = 0.043). The time taken to achieve a CD4 T-cell count >500cells/ml despite long-term cART is prolonged in a subset of patients. Starting cART early with a PI-based regimen vs. NNRTI-based regimen is concomitant with more rapid recovery of a CD4 T-cell count >500cells/ml (49). Other study findings have found similar results, whereby lower CD4 outcome is related with lower baseline CD4 cell counts, longer ARV treatment duration and older (48–52).

Study done by Vemula et al., 2016, India reported that for those who were initiated ART therapy with base line CD4 count of < 350 cells/mm^3 the CD4 count was increased by a mean of 180.28 cells/mm3 after 6 month of ARV treatment use. In this study females were shown to have more improvement in CD4 count than males (53).

Study conducted in Peru to assess Predictors of CD4+ cell count response and of adverse outcome among HIV-infected patients receiving ARV treatment reported Patients with a lower CD4+ cell count at baseline and those starting ARV treatment with a didanosine-based regimen had a higher increase in CD4+ cell count at six months. Whereas, Patients starting ARV treatment with a stavudine-based regimen had a lower increase in CD4+ cell count at six months (54).

Another Longitudinal CD4 T cell count was investigated in 293 participants of the Swiss for 5 years. Determinants of incomplete responses (CD4 count <500 cells/mL) were Older age (aOR, 1.71; 95% CI, 1.21–2.43), lower baseline CD4 T cell count (aOR, 0.37; 95% CI, 0.28–0.49), and longer duration of HIV infection (aOR, 2.39; 95% CI, 1.19–4.81) were significantly associated with a CD4 T cell count <500 cells/mL at 5 years (55).

A cross-sectional study was conducted on HIV-infected pre-and post-antiretroviral treatment, North West Ethiopia indicated the median of CD4 count before ART was 490 cells/mm^3 with an IQR of (286, 765); this increased to 663 cells mm^3 with an IQR of (499, 908) after ART (56).

A retrospective cohort study was conducted to examine the predictors of CD4 count among ART users, Northwest,Ethiopia revealed that the mean rates of incensement of CD4 counts for patients with ambulatory/bedridden and working baseline functional status were 17.4 and 30.6 cells/mm3 per year, respectively. For each additional baseline CD4 count, the gain in CD4 count during treatment was 0.818 cells/mm3 ($p < 0.001$). In this study, higher baseline CD4 count, younger age, working functional status, and time in treatment contributed positively to the increment of the CD4 count. However, the observed increment at 4 years was unsatisfactory as the proportion of ART users who reached the normal range of CD4 count was very low (57).

3. Objectives

3.1 General objective

To assess immuno-hematological parameters among adult HIV positive patients

Before and after initiation of 1j HAART at St. Peter specialized Hospital, Addis Ababa, Ethiopia, 2021.

3.2 Specific objectives

1. To assess hematological parameters among Adult HIV positive patients before and after initiation of 1j HAART regimen at St. Peter specialized hospital, Addis Ababa, Ethiopia, 2021

2. To assess CD4+outcomes among adult HIV patients before and after initiation of 1j HAART regimen at St. Peter specialized Hospital, Addis Ababa, Ethiopia, 2021.

4. Materials and methods

4.1 Study area

The study area was St. Peter specialized Hospital which is one of federal referral hospital in Addis Ababa, Ethiopia. The Hospital is located in Gulale sub city. It was established in 1953 as public Hospital. The Hospital serves as specialized Hospital with more than 300 beds and gives different inpatient and outpatient services to populations in the surrounding area and the adjacent regions. At the time of study period, the Hospital provided ART service for about 2367 HIV patients as supported by counseling, caring and routine laboratory monitoring (58).

4.2 Study design and period

Prospective institution based cross-sectional study was conducted from May 1 to July 30,2021.

4.3 Population

4.3.1 Source of population

All HIV positive patients attending at St. Peter Specialized Hospital.

4.3.2 Study population

Adult HIV infected individuals enrolled for ARV at St. Peter Specialized Hospital.

4.4 Eligibility criteria

4.4.1 Inclusion criteria

1. HIV patient on ARV treatment for more than three months and records are legible and complete and who have complete baseline Blood Count (CBC) and CD4 count.
2. HIV patient who showedwillingness to participate in the study and give informed consent for enrollment.

4.4.2 Exclusion criteria

1. Patient under the age of 18 years
2. HIV patient with chronic disease such as TB
3. Pregnant women
4. HIV patient who had poor adherence or interrupted the medication
5. HIV patient with underlying hematological malignancies

4.5 Study variables

4.5.1 Dependent variables

-Hematological Profiles

-CD4count

4.5.2 Independent variables

- Socio-demographic characteristics

-ARV drug

-Base Line (CBC and CD4 count)

-Duration use of ARV treatment

-WHO Clinical stage

4.6 Measurement and data collection

4.6.1 Sample size determination

Sample size was estimated using a single population proportion formula, since there is no previous study done on the ARV TREATMENT based Hematological outcome, taking expected prevalence rate of anemia (P = 50%) and5% level of precision (d) with 95% confidence interval.

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

$$n = \frac{1.96^2 * 0.5(0.5)}{0.05^2}$$

$n = 384$andby considering 10% non-responsive rate,

Then the final sample sizes became 422 HIV patients on ART at St. Peter Specialized Hospital.

4.6.2 Sampling method

Convenient sampling method was used

4.6.3 Data collection procedure

Socio-demographic data

Data collection was based on the indicated eligibility criteria. Structured questionnaire was constructed composed of age, sex, income status, and marital status), WHO clinical stage, opportunistic infections (OIS), other co-morbidity and ART duration.

The principal investigator (PI) and all Health care providers of ART unit involved in the data collection process of the study.

4.6.4 Specimen collection

About 5ml of venous blood was collected usingK_2EDTA test tube with labeled double ID by senior technologist for hematologic profiles (CBC) and CD4 count from each individual who had signed consent and interviewed.

4.6.5 Laboratory testing principles

The Beckman Coulter method is based on the principle of impedance method. As each cell pass through the aperture, it momentarily increases the resistance of electrical path between the submerged electrodes on either side of the aperture which then causes a measureable electronic pulse. The number of the pulses indicates cell count whereas the size of the electrical pulse generated is relational to volume of the cell. The hemoglobin is photo metrically measured at 525 nm. The lytic reagent rapidly and simultaneously destroys the RBC and converts Hb into stable pigment, which is proportional to the concentration of Hb. VCS technology is used to count the WBC differentials. Analysis and classification of WBCs use three simultaneous measurements of individual cell volume (V), high frequency conductivity (C), and laser light scatter (S).

For, CD4 count, BDFACS presto was used and assay principle is that when blood is introduced into the BD FACS Presto™ Cartridge, the specific antibodies bind to the surface antigens on the T lymphocytes. When the stained cartridge is inserted into the counter, the dedicated software identifies and counts the CD4+ T lymphocyte. The BD FACSPresto™ Cartridge also contains dried fluorochrome conjugated antibody reagents and immobilized antibodies as a quality control measure which the instrument uses to ensure that the reagents are present and sufficient blood specimen volume has been added.

4.7 Data collection tools and procedures

4.7.1 Data quality assurance

To assure the quality of data, training was given to the data collectors. Standard operating procedure was strictly followed during specimen collection and laboratory procedures. A pre-tested, structured questionnaire was prepared in English and translated to local language, Amharic to obtain more reliable information. Completeness of data collected was checked before analysis of data.

4.7.2 Laboratory parameters quality assurance

Pre analytical

Blood sample was collected by experienced laboratory technologist from each participant for Hematologic parameters (CBC) and CD4 and the required sample storage condition will be maintained during sample processing.

Analytical

The quality and performance of hematology analyzer was checked by running quality control samples along with the patients' sample. To maintain the quality of parameter test, the manufacturer's instructions (manual) for the Beckman Coulter (DxH800) hematology analyzer (59) and SOPs for test parameters was strictly followed and every procedure was performed by experienced laboratory personnel. The three controls levels; Low, Normal and High levels for CBC, from Coulter Hematology analyzer manufacturer along with patients' samples was run to obtain the full complete blood count.BD FACS PRESTO instrument quality control feature automatically checks counting accuracy at the beginning of the day self-test.

Post Analytical

The data obtained was then interpreted, stored to computer and data access was limited and locked by password to keep the confidentiality of these data. Leftover samples were disposed as per the laboratory safety protocol of Hospital.

4.8 Data analysis

After the collected data was cleaned and verified it was analyzed using SPSS version 21.

Appropriate statistical methods like t-test (paired), to compare differences in hematological outcome before and after ARV therapy and Logistic regression analysis was applied to determine the associations of established risk factors for immunologic and hematologic abnormalities. Statistical significance was considered at $p< 0.05$ with 95% CI.

4.9 Ethical Approval

Ethical clearance was obtained from Department of Medical Laboratory Sciences Research and Ethics Review Committee College of Health Science, Addis Ababa University and Addis Ababa Health Bureau Ethical and Research committee (AAHBREC). Addis Ababa public health research and emergency management directorate. Written informed consent was sought from all participants before enrollment. Any information obtained from the participants was strictly confidential. Unique Subject identification number (SID) was used as identification for each study subjects.

4.10 Dissemination of results

The findings of this study will be presented at the Department of Medical Laboratory Sciences of Addis Ababa University and copies will be shared with St. Peter Specialized Hospital. The findings will be also published on the international journals.

4.11 Operational definitions

ARV treatment is receipt of two nucleoside reverse transcriptase inhibitors (NRTI) and one non-nucleoside reverse transcriptase inhibitor (NNRTI) or one protease inhibitor (PI)and which are given as first line drug

Hematological Profiles are white cell counts and differential white cell counts, RBC Count and indices and total platelet count and indices.

Immunological Parameter is CD4count

Hematological indices-are defined as red blood cell indices (MCV, MCH, MCHC, and RDW) and platelet indices (MPV, PCT, PDW).

Immunosuppression is defined based on the CD4 count <500 cells/µl as per the WHO guideline.

Hematological abnormalities are rearrangements of hematological parameters.

Anemia, Leucopenia, Neutropenia, and thrombocytopenia were defined based on established St.peter Specialized Hospital laboratory reference range and according to WHO Hb cutoffs. Accordingly, for females Hb concentration of 12 g/dL or higher was considered normal and anemic for Hb concentration< 12.0 g/dl (11.0–11.9 g/ dL =mild, 8.0–10.9 g/ dL = moderate, and < 8.0 g/dL = severe) and for males Hb concentration cut of 13 g/dL or higher was considered normal and anemia was defined as Hb concentration <13 g/dL (11.0–12.9 g/dL as mild; 8.0– 10.9 g/ dL as moderate, and < 8.0 g/dL as severe) (60).

Platelet count of 150-450×10^3/mm^3 is considered normal and Thrombocytopenia is defined as total platelet count < 150 × 10^3/mm^3 and Leucocyte count of 4-11× 103/mm3 was considered normal and leucopenia is defined as total WBC count < 4 × 10^3/mm^3.

5. Results

5.1 Socio demographic Characteristics of Study participants

Of 422 Adult HIV patients on ARV treatment studied, the median age of study subjects was 42 years with interquartile range (IQR) of (35, 49). About273 (64.7%) were females. The majority, 255(60.4%), 183(43.4%) were low by income status and married by marital Status, respectively. Detailed socio-demographic characteristics are shown in Table 1.

Table 1: Socio-demographic characteristics of HIV patients on ARV treatment at St.Peter Specialized Hospital, Addis Ababa, Ethiopia, 2021(N=422).

Variables	Category	N(%)
Age (year)	18-34	81(19.2)
	35-45	199(47.2)
	>45	142(33.6)
Sex	Male	149(35.3)
	Female	273(64.7)
Marital status	Single	66(15.6)
	Married	183(43.4)
	Divorced	112(26.5)
	Widowed	61(14.5)
Income status*	Low(<500ETB)	255(60.4)
	Medium(3001-5000ETB)	127(30.1)
	High(>5000ETB)	40(9.5)
Educational Status	Illiterate	77(18.2)
	Primary	154(36.5)
	High School	128(30.3)
	Certificate and above	63(14.9)

ETB=Ethiopian Birr

* Income status category was based on EDHS,2019

5.2 Baseline features of study subjects

Before shifted to 1j based ARV treatment, the majority (64.7%)of study participants were initiated on TDF based ARV treatment regimen and majority of study subjects were at WHO clinical stage I and II 269 (63.7%) (table2).

Table 2: Baseline clinical characteristics of HIV patients after ARV treatment at St.Peter Specialized Hospital, Addis Ababa, Ethiopia, 2021(N=422).

Variables	Category	N(%)
WHO stages	I-II	269 (63.7)
	III-IV	153(36.3)
ARV treatment type	AZT based	149(35.3)
	TDF based	273(64.7)
CD4 count (cells/μl) (mean±SD)	435.56± 235.5	
Hb(g/dl)(mean±SD)	14.3±2.69	
ARV treatment duration (months) (mean±SD)	12.07± 6.25	

Key: Hb=Hemoglobin, SD=Standard Deviation

5.3 Immuno-Hematological parameters outcome among study participants after ARV treatment

Paired T-test was applied to realize the mean variation of Immno-hematological outcome among study participants after ARV treatment. After shifted to 1j ARV treatment for >3 months the mean White Blood cells, RBC, Hb, PDW, CD4, as well as Lymphocyte, Neutrophil and Platelets were increased significantly($P<0.05$). However, RDW and MCH were decreased ($P<0.05$).

5.3.1 White Blood Cells and WBC differentials outcome after ARV treatment

The mean WBC count after ARV treatment was significantly increased compared to the mean before ARV treatment initiation, shown in the above table 3. Thus, the change was evaluated by stratifying in to four duration category. Accordingly, the mean WBC >18months (highest) >6-18 months (Mean=$5.x10^3/mm^3$)>6 months (the least) recorded in indicating that the mean WBC increases with increasing duration on ARV treatment (table 4).

From current study,the mean % LYM of the study participants significantly increased after a minimum of 3 months ARV treatment initiation (P=<0.001). However, when the mean % LYM count was further compared based on after strafying the four ARV treatment duration category ,it is not significantly vary(P=0.057).

Moreover, the mean Neutrophil was also significantly increased after three months of ARV treatment as compared to before ARV treatment (p<0.001). Table 3. Moreover, there was significant variant mean change observed based on duration of ARV treatment usage (p=.020). indicating that the improvements in mean Neutrophil, over time.

5.3.2 Red blood cells and Hemoglobin concentration outcome after ARV Treatment

As shown in the Table 3, there was significant (P<0.05) change in the mean RBC following ARV treatment initiation. Furthermore, following ARV treatment, the mean hemoglobin levels (g/dl) was also significantly increased as compared to the value before treatment initiation (p<0.001). However, when stratified based duration on ARV treatment ,ir does not reach significany level in the mean hemoglobin levels (g/dl) between the ARV treatment duration category (P=0.086),table 4.

5.3.3 Platelet outcome after ARV treatment

The mean$\pm$SD of platelet count of the study participants were,250.65$\pm$70.14$\times10^3/\mu$l before starting ARV treatment and significatly increased to 260.14$\pm$ 89.93 $\times10^3/\mu$l after ARV treatment (p <. 0001) as shown in table 3. In addition, as illustrated in table 4 this mean increment in platelet count across ARV treatment category was significantly different between groups (p=0.026).

Table 3: Mean ± SD of Immuno-Hematological parameters of adult HIV patients before and after ARV treatment at St. Peter Specialized Hospital, Ethiopia.

Parameters	(mean± SD) values		P-value
	Before ARV therapy (422)	After 3Months on ARV therapy(422)	
CD4 count (cells/µl)	435.56 ±235.50	539.63 ±236.53	<0.001
WBC(10^3/mm^3)	5.2 ±6.29	6.43±3.59	<0.001
RBC(10^6/mm^3)	4.35±0.72	7.9±0.47	<0.05
Hb(g/dl)	14.43±2.69	15.1±2.68	<0.05
HCT (%)	42.8±2.09	45.1±6.97	0.15
PLT(10^3/mm^3)	250.65±70.14	260.14± 89.93	<0.05
LYM (%)	33.13±12.58	35.82±11.42	<0.001
MON (%)	5.44 ± 3.32	7.98±3.2	<0.001
NEU (%)	50.64± 13.01	56.65±12.25	<0.001
MCV(fl)	96±13.02	94.28±9.68	0.38
MCH(pg)	35.66±5.4	31.65±2.77	<0.05
MCHC(g/dl)	34.42±2.08	36.45±2.6	0.11
RDW(%)	17.11±11.52	14.74 ±9.66	<0.05
MPV (fl)	9.90 ±1.62	9.78 ±6.79	0.901
PDW (%)	12.38±6.13	14.67±1.98	<0.05

As depicted in table 4, there is significant differences for some of hematological parameters based on the duration of ARV treatment. Whereas, there is no significant difference in CD4 based on duration of ARV therapy.

Table 4: Mean ± SD of Immuno-hematological parameters among adult HIV infected individuals after ARV treatment, with respect to duration St.Peter Specialized Hospital, Addis Ababa, Ethiopia, 2021.

Parameters	Before ARV treatment	After ARV treatment				F	P
		Duration on ARV treatment					
		3-6 Months (n=83)	6-12 Months (n=97)	12-18Months (n=106)	>18Months (n=136)		
CD4(cells/ µl)	435.56±35	534.3±243.3	555.13±360.9	581.10±156	511.82±63	0.43	.725
WBC $x10^3/mm^3$	5.2 ±6.29	5.75±4.78	6.30±1.54	6.42±1.62	7.1±5.7	2.73	.043
RBC $x10^6/mm^3$	4.35±0.72	8.50 ±41	12.59±59.9	5.08±2.7	4.63±.61	1.10	0.34
Hbgm/dl	14.43±2.6	16.25±11.03	14.29 ±1.61	15.02±2.26	14.62± 2.1	2.21	.086
HCT %	42.8±2.1	44.38 ±4.48	42.53 ±4.6	44.17±5.41	45.94± 5.3	1.52	.207
PLT $x10^3/mm^3$	260.1±89.9	238.74±65.1	266.82±67.53	250.66±73.1	247.79±73	3.11	.026
LYM %	33.1+12.58	28.92±10.37	30.34±13.20	30.03+9.97	35.1±11.5	2.52	.057
MON %	5.44 ± 3.32	8.274±4.39	8.274±2.16	8.274 ±3.23	8.274±2.64	1.67	.171
NEU%	50.64±13.0	53.13±14.20	55.81±12.27	57.57±14.06	58.4±12.5	3.31	.020

5.4 magnitude of Hematological de arrangements after ARV treatment

From current study Anemia (24 %vs12.1%) and Leucopenia (30.5 % vs11.3%) were the most abnormality found before and after ARV treatment, respectively. Moreover, Majority of Anemic Patients Were Mild anemic (10.4%).

The changes in abnormalities were remarkable for improvements of anemia, leukopenia, thrombocytopenia and improvement of Immunosuppression in those who have been after ARV treatment for at least three months years (Figure 1).

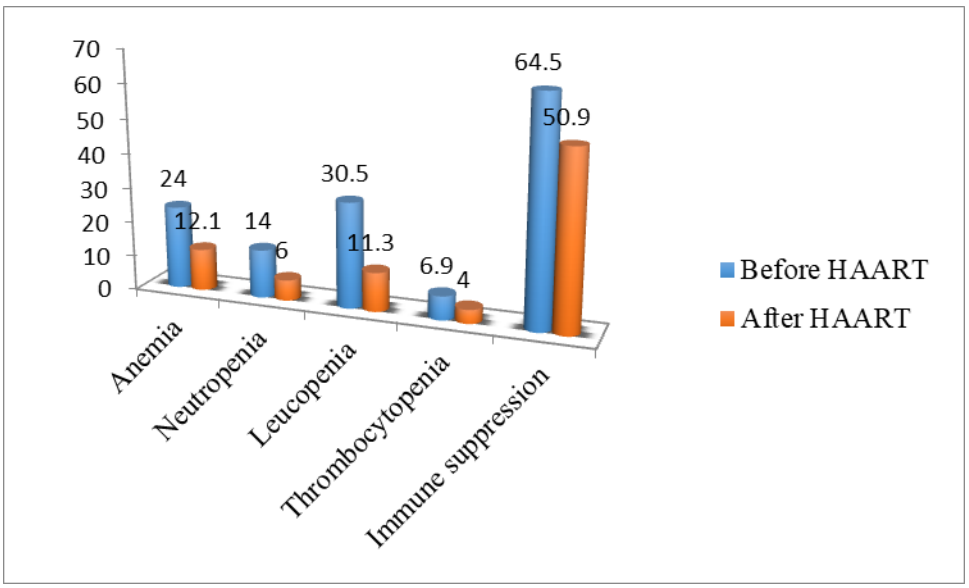

Figure 1: Common Immuno-hematological abnormalities among HIV patients after ARV treatment at St.Peter Specialized Hospital, Addis Ababa, Ethiopia, 2021.

5.4.1 Anemia and its predictors among adult patients after ARV treatment

From current finding, predictors for anemia were sex (females), WHO stage (III/IV) and CD4count (<200) (table 6). patient with WHO clinical stage three and four had 16 times increased odds of having anemia than those with stage one and two (AOR=16,95%CI:10.63-66.46, P=0.01), table 6.

Table 5: Predictors of anemia among study participants after ARV treatment at St.Peter Specialized Hospital, Addis Ababa, Ethiopia, 2021.

Covariates	Anemia		AOR (95%CI)	P-value
	Yes(n=51)	NO(n=371)		
Sex : M	12(2.8)	137((32.46)	1	
F	39 (9.2)	234(55.5)	7.8(1.9-32.2)	.004
Age : 18-34	9(2.1)	72(17.1)	1	0.37
35-45	29(6.9)	170(40.3)	1.1(.43-2.79)	.83
>45	13(3.1)	129(30.6)	.61(.30-1.56)	.36
Duration on ARV treatment	51(12.1)	371(87.9)		
Base Line CD4 +	5(1.2)	354(83.9)	1	
	46(10.9)	17(4%)	32 (46.3-98.5)	.0001
WHO Stage: I-II	8(1.9)	261(61.8)	1	
III-IV	43(10.2)	110(26.1)	16(10.63-66.46)	.001

5.5 CD4outcome after ARV treatment and its associated factors

In this study,the associated factors of Immune suppression after at least three months of ARV treatmentusage were: sex, base line CD4+ count and Income status of the participants. Female participants had 2.65 times increased risk of immune-suppression than males after at least 3 months of ARV treatment initiation (AOR=2.65,95%CI:1.47-4.79, P=0.01). The study also revealed that, participants with <200 baseline CD4 count had 10.8 times increased risk of immune-suppression after at least 3 months of ARV treatment (AOR=10.8,95%CI: 4.67-24.67, P=0.001) as compared to baseline CD4 count ≥500. Similarly, participants with baseline CD4+ count between 200 to 349 had 3.27 increased odds of immune-suppression after at least 3 months of the treatment (AOR=3.27,95%CI: 1.43-7.47, P=0.05) than those with baseline CD4 count of ≥500.

Finally, participants with low (AOR=6.2,95%CI:1.96-19.95, P=0.02) and medium (AOR=2.5,95%CI:1.31-4.77) income had 6.2 and 2.5 times, respectively increased odds of immune-suppression after 3 months of treatment than those with high income (Table 7).

Table 6: Predictors of immune suppression after ARV treatment among adult HIV patients at St.Peter Specialized Hospital, Addis Ababa, Ethiopia, 2021.

variables	Immune-suppression		Uni-variate		Multivariable	
			COR(95%CI)	P-value	AOR (95%CI	P-Value
	Yes (%)	No (%)				
Sex: Male	93(22)	56(13.3)	1			
Female	122(28.9)	151(35.8)	2.05(1.36-3.09)	0.001	2.65(1.47-4.7 9)	.001
Age : 18-34	41(9.7)	40(9.5)	1	.31	-	
35-45	101(23.9)	98(23.2)	1.6(.810-3.313			
>45	73(17.3)	69(16.4)	1.75(.809-3.78)	-		
Base line CD4 category						
≥500	29(6.9)	122(28.9)	1			
350-499	46(10.9)	50(11.8)	.8(0.39-1.78)	.638	-	
200-349	90(21.3)	21(5)	3.8(1.89-7.93)	.000	3.27(1.43-7.47)	.005
<200	50(11.8)	14(3.3)	15(7.33-30.793	.000	10.8(4.67-24.67)	.001
Income Status						
High	7(1.7)	20(4.7)	1			
Medium	43(10.2)	61(14.5)	3.74(1.53-9.12	.004	2.5(1.31-4.77)	.005
Low	165(39.1)	126(29.9)	1.85(1.18-2.92)	.007	6.2(1.96-19.95)	.002

Income status is based on EDHS,2019

6.Discussion

This study investigated the Immuno-hematological outcomes of adult 422 HIV patients on newly initiated ARV treatment at St.Peter Specialized Hospital. After at least 3 months of the ARV treatment use many hematological parameters; WBC, RBC, Hb, PLT, %NUE, %MON, %LYM and CD4 have shown improvements. On the other hand, RDW and MCH significantly decreased after a 3 months of ARV treatment.

6.1 The hematological parameters outcome of patients after ARV treatment

This study showed that the white cell count improved after ARV treatment. this is in line with finding of Kaur *et al*., 2017(41). and study done in DBRH, North-East Ethiopia,2020,that identified significant increment in WBC (4.65±1.53 X 103/μL,Vs 5.58±1.82 X 103/μL) after initiation of 1j (TDF-3TC-DTG) treatment (31).However, contrary to this finding, study done in Hematological abnormalities of adult HIV-infected patients before and after initiation of highly active antiretroviral treatment at Debre Tabor comprehensive specialized Hospital, Ethiopia indicated that a decrease in the mean WBC (6.5±2.4 10^9/L vs5.1±1.2$10^9$/L) compared to the before ARV treatment initiation(40).Justification for the difference could be due to, subjects may had had infections that lead to increase in WBC before ARV treatment and after ARV treatment may normalize itself and also ARV treatment improved immunity minimizing the Possibility of individuals who initiated ARV treatment being infected by OIS (62).

Current study indicated that, compared to before ARV treatment initiation, the Neutrophils and Monocyte, had significantly increased after the initiation of1j(TDF-3TC-DTG) treatment. This similar with findings of study done in DBRH, North-East Ethiopia,2020, that identified significant increment in mean Neutrophils after initiation of 1j (TDF-3TC-DTG) (31) and Rome, Italy that showed there was an improvement in the Neutrophils count after receiving ARV treatment.This is in fact due the fact that after ARV treatment, there is improvement in immunity against OIS that suppress the bone marrow Neutrophil and Monocyte function and expression (32).

From current study, the mean Lymphocyte was also showed significant increased after a 3 months ART initiation. This is in line with study conducted in India (4) and in Rome (32). However, the it is contra indicated with other report (42,63). Difference in results might be due to Patients imitation on induced ARV treatment regimen.

From current study showed improvement in mean RBC, HCT, MCHC and decreased RDW after ART initiation from before ARV treatment. In similar with this study, other studies also indicated improvements in RBC, MCV, MCH and MCHC and decreased RDW after ART initiation (18,31,41). Justification for this could be, the positive feedback of the ARV treatment that help in the decline of viral load, decreased release of immature Red belood cellls, decreseased desstruction Red blood cells and improved EPO response (35). However, this finding is contradicted with a finding of by Damtie et al., 2021, Ethiopia (40) and Iran (31). The difference from the current study findings is might be due to the difference in definition of RBC, study population and duration on ARV treatment and difference ARV treatment type.

In this sudy, the mean platelet was 250.65+70.14x10^3/μl before ARV treatment and 260.14+ 89.93x10^3 /μl after receiving ARV treatment. In line with this other studies also reported incremental improvement in PLT after ARV treatment initiation(31,43–46). This might be due to the reverting of Thrombocyte abnormalities to normality after the initiation of beneficial effect of active ARV treatment in reducing the frequencies of thrombocytopenia (38,45).

6.2 Prevalence of Hematological Abnormalities among Adult HIV Patients after ARV treatment

From this finding, the magnitude of anemia was found to be 24 %before ARV treatment initiation and 12.1 after ARV treatment initiation of which about 10.4% were mild anemic. Moreover, the predictors of detected anemia were, Sex (female) and Low CD4 (<200count). This concordant with the findings of Harris et al.,in 2008, across the Europe that revealed the magnitude of anemia before and After ARV treatment initiation was 35 % and 26% with mild(21.4%),moderate(3.7%) and severe (0.4%) and was associated with Sex (higher females) and CD4 count(increased with decreasing CD4) (27).Other studies also indicated Anemia is associated with, Low CD4 count(<200), Advanced clinical stage (III/IV)of HIV (12,17,18,28,37).The possible justification for this might be ;the decreased Anemia after ARV treatment is due to the positive effect of active ARV treatment in reducing the frequencies of Anemia, and also the positive feedback of the ARV treatment that help in the decline of viral load, decreseased desstruction red blood cells either by vira infection,supperssiion by OIS that lead to Anemia and the detected anemia are due to in sever immunosuppression (low Cd4 count) and advanced HIV stage ,patient are at risk of developing OIS and High Viral load which in turn induce anemia (62).

In curent Thrombocytopenia was noticed in about 6% before ARV treatment and 4 % after ARV treatment. This is in line with study conducted Study conducted in Beijing Ditan

Hospital, China in 2021 on Thrombocyte Abnormalities in HIV-Positive Patients Before and After the Initiation of ARV treatment showed the prevalence was 2.65% among ART-naïve patients(38) and Addis Ababa, Ethiopia which showed the prevalence of after ARV treatment was thrombocytopenia 4.1% (47).Explanation for this could be the reversion of the reduction of the noted thrombocytopenia due to reduction of infection of megakaryocytes by the HIV virus after ARV treatment, reduction of immune-mediated destruction of platelets by antibodies, cross-reacting antibodies that are targeted against HIV proteins, elimination of OIS that Couse marrow suppression that lead to Thrombocytopenia after effective ARV treatment (25). Contrary to the present study, a study conducted in Japan reported that the severe thrombocytopenia during DTG-containing antiretroviral therapy. After initiation of dolutegravir (DTG) the platelet count was decreased to ≤50,000/μL. This could due to megakaryocytes or a direct infection of megakaryocytes by HIV virus or co-infection with another virus, opportunistic infections and myelosuppression effect of medication (64).

From the current study, the prevalence of Leukopenia 30.5% before ARV treatment and 14% after ARV treatment. This finding is concordant with a Study conducted by Thulasi *et al.*, 2016 showed the magnitude and severity of leucopenia was found to be less in patients after ARV treatment (29). However, Contrary to current finding study conducted in Ghana reported the prevalence of leukopenia showed an increasing pattern after initiation of ARV treatment (36). This abnormality could have resulted from the use of a zidovudine-containing ARV treatment regimen, which can cause leukopenia by suppression of bone marrow production and cytotoxicity of T-cells eventually decreasing the survival of T-cells. But, in this study, Leukopenia is decreased by more than 50% after ARV treatment initiation. This may be because, difference in ARV treatment types other previous studies, that used first line of ART includes a combination of Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI's) and Nucleoside Reverse Transcriptase Inhibitors (NRTI's)based but the current study was based on integrase strand transfer inhibitors (INSTIs) or Dolutegravir (DTG).

In this study, Neutropenia was 11.3%, before ARV treatment and 6% after ARV treatment. In similar with this, study done in DBRH, North-East Ethiopia,2020, showed the magnitude of neutropenia was 7.0%, before initiation of ART and 1.1 after initiation of ARV treatment (42). Other findings have also reported the decrease in Prevalence of Neutropenia after ARV treatment (4,33,34). Increase in magnitude of Neutropenia before ARV treatment initiation is due to increased marrow suppression by OIs which in turn alter production neutrophil, decreased amounts of granulocyte colony-stimulating factor, due to HIV mediated suppression of the bone marrow with end products being neutropenia, Apoptosis, which

markedly is accelerated in neutrophils in patients with HIV infection, tending to shorten their survival and after which it reverted after initiation of ARV treatment (14). However, the present finding is contra indicated than the study of Gondar, Ethiopia that reported14.5% before ARV treatment and 28.3% after initiation of ARV treatment. The difference might be because of the difference in, ARV treatment used, the study population, and clinical condition (18).

6.3 CD4+outcome among HIV patients after ARV treatment

In this study, the mean cluster of differentiation 4 (CD4)+ T cell count was significantly increased (435.56±235.50 vs 539.63±236.53cells/mm3) before and after ARV treatment initiation, respectively. This in line with the Cross-sectional study done in North-East Ethiopia ,2020, that showed CD4+ T cell counts (161 ±106.5 cells/mm3 vs 381.2±190.9 cells/mm3) before and after ARV treatment initiation, respectively (42). Other studies have also reported the improvement in mean CD4 count after ARV treatment (40,53,56,57,65). The increment in mean CD4 count is due to the fact that the role of ARV treatment in the depletion of the viral load which in turn contribute to the immune recovery and efficacy of ARV treatment in improving the CD4 count even within three months of beginning treatment.

The predictors of detected immune suppression after at least three months of ARV treatmentusage were: sex, base line CD4 count<200 cells/$mm^3$and income status of the participants. Female participants had 2.65 times increased risk of immune-suppression than males after at least 3 months of ARV treatment initiation (AOR=2.65,95%CI:1.47-4.79, P=0.01). participants with <200 baseline CD4 count had 10.8 times increased risk of immune-suppression after at least 3 months of ARV treatment (AOR=10.8,95%CI: 4.67-24.67, P=0.001) as compared to baseline CD4 count ≥500. participants with low (AOR=6.2,95%CI:1.96-19.95, P=0.02) and medium (AOR=2.5,95%CI:1.31-4.77) income had 6.2 and 2.5 times, respectively increased odds of immune-suppression after 3 months of treatment than those with high income. This is consistent with study done by Vemula et al., 2016,India reported that for those who were initiated ART therapy with base line CD4 count of < 350 cells/mm^3 the CD4 count was increased by a mean of 180.28 cells/mm3 after 6 month of ARV treatment use .In this study females were also shown to have more improvement in CD4 count than males (53) and finding of Australia that reported a lesser but significant number of patients do not achieve CD4 T-cell counts >500cells/ml despite years of suppressive cART. Clinical factors associated with CD4 T-cell recovery following long-term cART, faster time to achieve a CD4 T-cell count >500cells/ml was associated with higher

baseline CD4 T-cell counts (p,0.001), younger age (p = 0.019) and treatment initiation with a protease inhibitor (PI)-based regimen vs. non-nucleoside reverse transcriptase inhibitor, NNRTI; (p = 0.043). The time taken to achieve a CD4 T-cell count >500cells/ml despite long-term cART is prolonged in a subset of patients. Starting cART early with a PI-based regimen vs. NNRTI-based regimen is concomitant with more rapid recovery of a CD4 T-cell count >500cells/ml (49). Other study findings have found similar results, where by lower CD4 outcome is related with lower baseline CD4 cell counts, longer ARV treatment duration and older age (48–52,55,65). However, this finding is contra indicated with study conducted in Peru to assess Predictors of CD4+ cell count response and of adverse outcome among HIV-infected patients receiving (ARV treatment) reported Patients with a lower CD4+ cell count at baseline and those starting ARV treatment with a didanosine-based regimen had a higher increase in CD4+ cell count at six months (54). The difference could be the difference in ARV treatment type or regimen and duration, Population characteristics.

7. Strength and Limitation of Study

7.1 Strength of the study

The main strength of this study was to assess the 1J based ARV treatment based Immuno-Hematological outcome. Moreover, these parameters can be used as markers for monitoring treatment purpose.

7.2 Limitation of Study

- Though the study was aimed to assess the Immuno-hematological out came the ARV treatment, it was limited by the absence of comparative hematological and CD4+ parameters outcome based on ARV treatment regimen type. Thus, it could not determine the comparative outcome of each type with ARV treatment.

8. Conclusion and recommendation

8.1 Conclusion

The mean CD4+, RBC, WBC count, Neutrophil, Platelets, Hb, LYM and HCT significantly increased among study participants. Whereas RDW and MCH significantly decreased after 3 months of ARV treatment. Moreover, current finding indicated the mean WBC, LYM% and PLT counts after ARV treatment increases with increasing duration on ARV treatment.

The predictors of Immune suppression of CD4 count <500 cells/µl after at least three months of ARV treatment were; sex, higher in women than in men (AOR 2.65 ,95% CI: 1.47-4.79, p <0.001), Base line CD4 count, higher in those with base line CD4 count<200(AOR 10.8,95%CI:4.67-24.67, p<0.001) and those having low income (AOR 6.21 95%CI:.96-19.95, P<0.01).

8.2. Recommendation

Based on the current study the following recommendations were made:

- Since, the ARV treatment initiation benefits the HIV patients of life individuals have to initiated this ARV treatment as early as possible as per national program me.Additionally, the current study found out only Two years usage of 1j ARV treatment, the long term Immuno-Hematological parameters outcome needs further investigation.
- To insure better management and to attain a better quality life of HIV patients, Health care providers should initiate patients ARV treatment of choice with consideration of Immuno-Hematological parameters, as surrogate markers for monitoring treatment response. This better improves many CD4+ and Hematological parameters.
- Further study by ARV regimen type is recommended.

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Annexes: Information sheet

Annex 1: consent form (English version)

RESEARCH TITLE: Assessment immuno-hematological parameters among adult HIV positive patients
before and after initiation of 1j HAART at St. Peter specialized Hospital, Addis Ababa, Ethiopia.

NAME OF MAIN RESEARCHER: Ayantu Gudina (BSc, MSc candidate)

NAME OF ORGANIZATION: Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University

INTRODUCTION: You are invited to participate as a study subject in a research conducted by MSc candidate, from Addis Ababa University. Your participation is voluntarily. The research teams are one principal investigator, two advisors from Addis Ababa University. Please take as much time as you need to read or listen in the information sheet.

PURPOSE OF THE RESEARCH PROJECT: We are asking you to take part in this study because we will try to assess hematological andCD4+parameter among adult HIV positive patient before and after initiation of ARV treatment.

You will not be given any monetary benefits; neither will you incur any costs. The study will benefit you and the nation in those reliable and less toxic regimens will be identified in the study findings. Hence, a reduction of morbidity and mortality due to HIV burden and drug toxicity. The results will be disseminated to the management of St. peter Specialized Hospital and the AA Health Bureau for evaluation.

PROCEDURE AND EXPECTED PARTICIPATION: If you are willing to participate, you need to understand the purpose of the study and give your consent. Not only this but also specimen collected from you will be used for the research purpose, and the results of your sample will be exposed to some concerned professional staffs as it is needed. The required sample will be collected by residents of hematology department. Then, you are requested to give your consent to the sample collector. After consent, a sample will be taken from venous blood. Moreover, there will be a face-to-face interview for additional questions.

POTENTIAL RISKS AND DISCOMFORTS: there might be some minimal risk and discomfort when we take venous blood. Nevertheless, we will try to minimize the discomfort as much as possible, as the blood samples will be taken by experienced laboratory professionals.

CONFIDENTIALITY

We respect your privacy and confidentiality. Any information that identifies you will not be shared with anyone else outside the study team. The information we will collect from you as part of the study will be kept in a locked file cabinet, or be protected by a password on the computer only accessible to personnel involved in the study.There is no sensitive issue that you will be asked related with your social desirability but any information that is obtained in connection with this study and that can be identified with you will remain confidential.

POTENTIAL BENEFITS TO SUBJECTS AND TO THE SOCIETY

You will not receive any payment for your participation in this research study as compensation. However, based on the diagnosis result you will be treated in view of that.

PARTICIPATION AND WITHDRAWAL FROM THE STUDY

The participation is voluntary and you have the right not to participate in this study. You may withdraw at any time and place without consequences of any kind. You may also reject to give any sample. You can ask any questions regarding to this study and you have a right to get a laboratory diagnosis result free.

CONTACT INFORMATION

If you have any questions about this study you can contact the following principal investigators and advisors for further information.

NameAyantuGudina (BSC), MSc student at the AAU,
Phone No. +251 9-47-72-53-45
Email:keyeron2018@gmail.com
Advisors/Co-investigators: Fikadu Urgessa(Bsc,MSc, Phd candidate) and Moges Hordofa (Bsc,MSc,)
Name of institute: AAU
Reviewed by: DREC (AAU), AAREC

Annex II. Consent form for adults (>18 years)

Consent form to participants on the study of assessment of immuno-hematological parameters among adult HIV positive patients before and after initiation of 1j HAART at St. Peter specialized Hospital, Addis Ababa, Ethiopia.

I have read the information above, or it has been read to me. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction. I voluntarily consent that I would participate in this study.To collect my blood and be a participant in this study and understand that I have the right to withdraw from the study at any time . My questions concerning this study have been answered.I know that there is no special payment for being participating in the study.I agree to take part in this study.

Name: _____ Signature: _____ Date: _____

If illiterate; Print name of independent literate witness, date and signature of witness (if possible, this person should be selected by the participant and should have no connection to the research team)

Name: _____ Signature: _____ Date: _____

We thank you for consenting to take part in the study

Annex III: Questionnaires (English version)

ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES.

TOOLS FOR QUESTIONARIE

Study title: Assessment of immuno-hematological parameters among adult HIV positive patients before and after initiation of 1j HAART at St. Peter specialized Hospital, Addis Ababa, Ethiopia, from May to July 2021.

Introduction: I am Ayantu Gudina. I am master's student in Hematology and immunology track at the Addis Ababa University. I am conducting a study on Hematology parameters outcomes on ARV treatment among Adult HIV patients. I am kindly requesting you to give me permission to use the residual specimen for my research. The information you provide will be used to improve health policy and help us to identify the most reliable and less toxic ARV regimen for your therapy. Complete confidentiality of your test results will be ensured.

Risk / benefits: You will not be given any monetary benefits; neither will you incur any costs. The study will benefit you and the nation in those reliable and less toxic regimens will be identified in the study findings. Hence, a reduction of morbidity and mortality due to HIV burden and drug toxicity. The results will be disseminated to the management of St. Peter Specialized Hospital and the Addis Ababa Health Bureau for evaluation.

Participant's rights: Your participation in this study is voluntary and if you decline to participate, you will not be denied any services that are normally available to you.

Confidentiality: You will be assigned a study code to link you from the Hospital number.

Records relating to you or your patient's participation in the study will remain confidential.

You will be given a signed copy of the consent form.

Contact information: If you have questions now or in the future regarding your rights or this study, you may contact AyantuGudina (BSC), MSc student at the AAU,

Phone No. +251 9-47-72-53-45

Email:keyeron2018@gmail.com

Code:_____

1. Socio demographic characteristics related questions.

SN	Questions	Response
101	Sex	1.Female 2. Male
102	Age (years)	_____
103	Marital status	1. Married 2. Single 3. Divorced 4. Widowed
104	Occupation	1)Civil servants 2) House wife 3) Private organization 4) Farmer 5) Daily laborer 6) Merchant
105	Educational status	1. Illiterate 2. Primary school 3. High school 4. Certificate and above
106	Is there any Co morbidities?	1.yes 2.No
107	If yes to No.106 above,which Co morbidity/ies it is?	1)tuberculosis 2)hepatitis B infection 3) other specify_____
108	Monthly Personal Income(in Birr)	_____
	2.Clinical and hematological related questions	

201	Regimen type	1. 1C=ZDV/3TC/NVP 2. 1D: ZDV/3TC/EFV 3. 1e= TDF/3TC/EFV 4. 1f= TDF/3TC/EFV 5. 1G=DTG/3TC/EFV 6. Other----------
202	ART duration(months)	_____
203	WHO clinical stage	a. Stage I b. Stage II c. Stage III d. Stage IV
204	opportunistic infections (OIs)	1. Chronic GE 2. Pneumonia 3. Candidiasis 4. Other Specify_____
205	Base line Hematological and CD4 count	1.WBC_____$10^3/mm^3$ LYM____%MON____ % GRAN ____ % 2.RBC_____ $10^6/mm^3$HGB____g/dl HCT____ % MCV____ μm^3 MCH_____pgMCHC___g/dl RDW____% 3.PLT_____$10^3/mm^3$ MPV_____μm^3 PCT_____% PDW____% 2.CD4 count_____

1e= TDF/3TC/EFV 1f= TDF /3TC/ EFV, 1C=ZDV/3TC/NVP 1G=DTG/3TC/EFV

Annex IV: **Annex IV: Amharic version informed consent**

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Annex V: Amharic Version

መለያቁጥር _____

ተቁ	መጠየቆች	መልስ
ክፍል1፡ማህበራዊ ሁኔታ		
101	ፆታ	ሀ)ወንድ ለ) ሴት
102	እድሜ	_____
103	የጋብቻ ሁኔታ	ሀ) ያገባ/ባች ሐ) የፈታ/ች ለ) ያላገባ/ባች መ) በሞትየተለየ
104	የስራ ሁኔታ	ሀ) መንግስት ሰራተኛ 2) የቤት እመቤት 3) የግል 4) ገበሬ 5) የቀን ሰራትኛ 6) ነጋዴ
105	የትምህርት ደረጃ	ሀ) ያልተማረ/ች ሐ) ሁለተኛ ደረጃ ለ) አንደኛ ደረጃ መ) ሰርትፍኬት እና ከዚያ በላይ
106	ሌላ አይነት ህመም አሞክ/ሽ ያውቃል?	1.አዎ 2.አይ
107	በተ.ቁ 106 መልስክ (ሽ) አዎ ከሆነ የትኛው	1.ቲቢ 2. ስኳር 3.ሄፕታይተስ B አንፈክሺን 4.ሌላ ካለ ይጥቀሱ----------
108	ወራዊ ገቢ (በብር)	----------
2. ክፍል 2፡የኤች አይ ቪ መድሀኒት ሲጀመሩ የነበሩ ባህርያት		
201	የሚወስዱት የመድሀኒት አይነት	1. 1C=ZDV/3TC/NVP 2. 1D: ZDV/3TC/EFV 3. 1e= TDF/3TC/EFV 4. 1f= TDF/3TC/EFV 5. 1j=DTG/3TC/TDF 6. ሌላ ካለ ይጥቀሱ ----------
202	የኤች አይ ቪ መድሀኒት ለምን ያህልጊዜ ወሰዱ(በወራት)	----------

203	የ WHO የኤች አይ ቪ መድሀኒት ሲጀምሩ የነበረዉ የበሽታ ደረጃ	ሀ) ደረጃ 1 ሐ) ደረጃ 3 ለ) ደረጃ 2 መ) ደረጃ 4
204	አስኒ ኣጋጣሚ ኢንፍክሽን ካለ	1. ስርየሰደደ የኣንጀት ኢንፈክሽን 2. የሳምባ ምች 3. ፈንገስ 4. ሌላ ካሌ ይጥቀሱ_____
205	የኤች አይ ቪ መድሀኒት ሲመጀሩ የነበረዉ የደም ምርመራ ዉጤት	1.WBC_____$10^3/mm^3$ LYM____%MON____ % GRAN ____ % 2.RBC_____ $10^6/mm^3$HGB____g/dl HCT____ % MCV____ μm^3 MCH_____pgMCHC___g/dl RDW____% 3.PLT_____$10^3/mm^3$ MPV_____μm^3 PCT_____% PDW____% 2. CD4 count_____

1e= TDF/3TC/EFV 1f= TDF /3TC/ EFV ,1C=ZDV/3TC/NVP. 1J=DTG/3TC/TDF

ስለ ተሳትፎዎ አመሰግናለሁ።

Annex VI: Laboratory principles and procedures

I. Laboratory reagents, supplies and equipment for CBC

1. 3 or 4 ml Purple vacutainer tube (EDTA)
2. Vacutainer holder
3. Alcohol swab
4. Cotton balls
5. Normal saline
6. Beckman Coulter (DxH 800) machine
7. Printer
8. Barcode Reader
9. Screen touch Monitor
10. UPS
11. Mixer
12. Tourniquet

II. Procedures of tests

1. Specimen was collected into EDTA (purple) vacutainer (3 or 4 ml volume).
This procedure was performed by strictly adhering to SOP and all expected safety rule will be applicable.
2. Check to see that the reagents needed for the number of the samples to be processed for the day are available.
3. Turn on the IPU switch and log on screen wasappear on the computer. Enter the user name and password.
4. Wake up/ Go online the main unit on the machine. Daily-check, auto rinse, temperature stabilization and background check was automatically performed, and the "READY LED turns on (ready for analysis) was appear

II. Procedures for CD4

BDFACS presto principle and interpretation

Assay principle: When blood is introduced into the BD FACS Presto™ Cartridge, the specific antibodies bind to the surface antigens on the T lymphocytes and monocytes during the incubation period. When the stained cartridge is inserted into the counter, the dedicated software identifies and counts the CD4+ T lymphocyte absolute and percentage cells, and

calculates the hemoglobin concentration. The BD FACSPresto™ Cartridge also contains immobilized antibodies as a quality control measure which the instrument uses to ensure that the reagents are present and sufficient blood specimen volume has been added.

Procedure

Invert the tube 10 times to mix the contents well.

use the pipet to obtain the specimen. Use one pipet per specimen.

Gently squeeze the bulb on the pipet to form a drop of blood on the tip of the pipet

Carefully dispense the specimen into the inlet port. Hold the cartridges by its ridges only.

Declaration

The undersigned declares that this thesis complies with the regulations of the University and meets the accepted standards with respect to originality and quality. PI also agrees to accept responsibility for the scientific ethical and technical conduct of the research project and for provision of required progress reports.

M.Sc. candidate: AyantuGudina (B.Sc.)

Signature: _____

Date of submission:10/1/2022

This proposal has been submitted with our approval as advisors.

Advisor: FekaduUrgessa (MSc,PHD candidate)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: MogesWordofa (BSc, MSc)

Signature: _____

Date: _____

Place: Addis Ababa ,Ethiopia.