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ORIGINAL RESEARCH

Study Of Effect Of Antiretroviral Therapy On Clinical Profile And Hematological Parameters In HIV Positive Patients

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Abstract
BACKGROUND:-Human immunodeficiency virus (HIV) infection is a state of profound immunodeficiency. HIV infection is a multisystem disease and is associated often with a wide range of hematological abnormalities, including impaired hematopoiesis, immune mediated cytopenias and coagulopathies, particularly in the later part of the disease. These hematological abnormalities include anemia, leukopenia, thrombocytopenia, and sometimes pancytopenia with variable bone marrow abnormalities. Here we study the hematological abnormalities and clinical profile in HIV positive patients before and after 3 months of ART. Hence all newly diagnosed HIV patients were investigated for hematological abnormalities as it has a significant impact on the clinical outcome and treated accordingly to reduce morbidity and mortality and improve the quality of life.
METHODOLOGY:- A comparison study was conducted in a tertiary care center Guru Gobind Singh Medical College & Hospital Faridkot, Punjab for a period of 18 months on 100 HIV positive patients. The patients admitted to the Department of Medicine, attended OPD and ART center with the diagnosis of HIV infection as per standard NACO criteria were included in the study. A detailed history, physical examination, and relevant investigations were conducted. Data were collected using a pre-tested proforma to meet the objectives of the study.
RESULT:-In total 100 HIV positive patients the mean age (years) was 37.90 ± 11.66 and males were more common than females .35.0% of the participants had infection of HIV by heterosexual route of transmission, followed by intravenous drug use, by needle injury, by unknown source and only one percent had from mother to child transmission. In clinical profile common symptoms were malaise 38%, followed by fever 27%, weight loss 27%, than females .35.0% of the participants had infection of HIV by heterosexual route of transmission, followed by intravenous drug use, by needle injury, by unknown source and only one percent had from mother to child transmission. In clinical profile common symptoms were malaise 38%, followed by fever 27%, weight loss 27%, rash 14%, oral thrush 13%, cough 11%, seizure 2%, lymphadenopathy 1% and diarrhea 1% before taking ART. In hematological profile anemia (46%) was the most common laboratory finding in the study, followed by thrombocytopenia (24%) and leucopenia (8%).A significant improvement was seen in hematological parameters and clinical profile after 3 months of ART. Non significant changes was observed in liver function test and renal function test in the study.
CONCLUSION:- The prevalence of anemia, leucopenia and thrombocytopenia was relatively more common in HIV infected treatment naïve patients as compared to those on ART.

INTRODUCTION

Human immunodeficiency virus (HIV) is a retrovirus that infects immune system cells and impairs their function, leading to opportunistic infections and tumors (1-3). Human immunodeficiency virus (HIV) infection is a state of profound immunodeficiency, leading to a spectrum of diseases ranging from acute syndrome seen with primary infection and prolonged asymptomatic state to an advanced disease or full-blown acquired immunodeficiency syndrome (AIDS) (4). Presently, there are two categories of HIV isolates: HIV-type 1 (HIV-1) and HIV-type 2 (HIV-2). HIV-1 is the primary cause of AIDS worldwide, and HIV-2 is only found in specific parts of Western and Central Africa. HIV is a member of the Retroviridae family Lentivirus genus that shares genetic similarities with humans (5). In addition, HIV-2 seems to be less pathogenic than HIV-1, and infection takes longer time to develop into AIDS(6). HIV-1 has A,B,C,D,F,H,J and K subtypes, among which subtype C is predominant in India, while HIV-2 has only 2 reported subtypes i.e, A and B (found mostly in West Africa), none of which are prevalent in India(7). The most

prevalent mode of transmission of HIV-1 is through sexual contact with heterosexuals. Other modes of transmission include parent-to-child (vertical) transmission, intravenous drug use, homosexuality, and blood transfusions(8). After entering the host, HIV spreads widely to cells and tissue, gradually damaging lymph node architecture and triggering the host's immune system to launch an attack on it using CD4+ T cells and CD8+ T cells, which are then eliminated by the virus, permitting unrestricted HIV replication and ultimately leading to the development of full-blown AIDS (9). In HIV-positive patients, the most important biomarkers of disease stage and progression are the CD4 count and HIV RNA concentration (10-12). However, prognosis can be predicted or influenced by additional factors (13).HIV-positive patients frequently experience hematological abnormalities, including anemia, neutropenia, and thrombocytopenia(14,15). Usually, anemia in an HIV infected patient is normocytic and normochromic and corresponds to a low reticulocyte count (16). Anemia can occur for a number of reasons. There are three processes involved in its pathophysiology : 1) reduced production of red blood cells (RBCs) due to opportunistic infections, direct effects of HIV infection, myelosuppressive medications, decreased erythropoietin production, and hypogonadism; 2) increased destruction of RBCs due to autoimmune hemolytic anemia, thrombotic microangiopathy, and disseminated intravascular coagulation; and 3) ineffective production of RBCs due to deficiencies in folic acid and vitamin B12.Nutritional deficiencies, such as vitamin and iron deficiencies are common in developing countries(17). Nucleoside analogs other than ZDV are not associated with anemia. However, by using lower dosages of zidovudine in conjunction with other non myelosuppressive antiretroviral medications such as protease inhibitors, the frequency of anemia has been greatly reduced. Zidovudine is usually associated with marrow toxicity, particularly with long-term treatment (18). HIV infection also causes thrombocytopenia and neutropenia. Chronic HIV infection is now a well-known cause of chronic immune thrombocytopenic purpura. The destruction of platelets by antibodies that are immune-mediated and cross-reactive with HIV proteins, specifically gp120 and p24, are among the hypothesized mechanisms that have been documented. This type of platelet destruction is called immune thrombocytopenic purpura, which is characterized by very low platelet counts with a normal hematocrit and white blood cell count (19,20). The most prevalent type of leukopenia in those with advanced HIV infection (10–30%) is neutropenia (19).HIV infection also shows impact on hematological indices like MCV, MCH, and MCHC in patients regardless of age, sex and ART(21).Henceforth, it is concluded that hematological parameters and clinical sign and symptoms are important monitoring tools for assessing HIV treatment and prognosis. Antiretroviral medication use may have a favorable or unfavorable impact on these parameters, depending on the choice of combination used. The majority of research have detailed the hematological characteristics of HIV-positive people prior to HAART however, to our best knowledge, there are only a few studies in India that assess the hematological profiles after receiving HAART. Hence, the aim of the present study is to assess the hematological profiles among HIV infected adult after starting of HAART and compare the mean differences of selected hematological profiles between baseline, and 3 months after initiation of HAART. That is why, we planned the present study

MATERIAL AND METHODS

A hospital based comparison study was conducted in Department of Medicine in Guru Gobind Singh Medical College and hospital,Faridkot for a period of 18 months.The study enrolled 100 newly diagnosed HIV positive patients who were presented in General Medicine OPD, IPD & ART center. confirmed to be HIV positive by ELISA **Aim:-** 1. To assess the clinical profile before antiretroviral therapy and after antiretroviral therapy in HIV patients.

2. To study hematological parameters hemoglobin (Hb), red blood corpuscle (RBC), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular (MCH), mean corpuscular hemoglobin concentration (MCHC), total leukocyte count (TLC), absolute lymphocyte count, platelets count, mean platelet volume and RDW before and after antiretroviral therapy in HIV patients.

3. To compare the clinical profile and hematological parameters before and after antiretroviral therapy in HIV patients

- Inclusion criteria:

  Newly diagnosed HIV positive patients ≥18 years

- Exclusion criteria:

HIV co-infection with hepatitis B & hepatitis C.

Patients with chronic kidney diseases, chronic liver diseases, malignancies.

Patients on cytotoxic drugs, autoimmune disorders.

Patients with history of previous blood or blood product transfusion within 3 months

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The study was conducted following the Helsinki Declaration. Written informed consent was obtained from each respondent and the study was carried out after approval from the Institutional Ethics Committee.All the participants were told about the purpose of the study and were ensured strict confidentiality.

Detailed history about occupation (truck driver, health care worker, etc.), mode of transmission (needle prick injury, I/V drug user, sexual transmission, mother - child) and about symptoms like fever, cough, shortness of breath, headache, weight loss, loss of appetite, dysphagia, epigastric pain, vomiting, generalized weakness, bleeding, chest pain, memory impairment, difficulty in moving limbs, burning micturition, rash and seizure episodes were taken from patients. Sign like pallor, lymphadenopathy, pedal edema, tachypnoea, tachycardia, temperature, wheeze, crepitation, hepatomegaly, splenomegaly, fluid thrill asterixis, drowsiness, shifting dullness, oral candidiasis, bronchial breathing, and motor weakness were also noted in patients enrolled in study. An investigation like peripheral blood cell count was performed using a 5 part cell automated hematology analyzer for hemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total and differential leukocyte count, Mean platelet volume, red blood cells(RBC), hematocrit (HCT) and platelet count, absolute lymphocyte count and baseline CD4 count were collected. Patient was put on ART, and after 3 months, their clinical profile and hematological parameters were analyzed. Data were entered into Microsoft Excel and analyzed using IBM-SPSS v.23 software (SPSS Inc., New York, USA). A significance level of $p < 0.05$ was considered statistically significant.

RESULTS

- There were 100 newly diagnosed HIV positive patients in the study who were not on antiretroviral therapy.
- Table 1 shows that majority of participants i.e 34% were in the range of 18-30 years with a mean age (years) of 37.90 ± 11.66.Males comprised 57% and females comprised 43% of the total 100 HIV positive patients enrolled in this study with a male to female ratio of 1.3:1. Majority of the participants had HIV infection by heterosexual route of transmission, followed by intravenous drug use, by needle injury, by unknown source and only 1% of had route of transmission from mother to child.
- The distribution of clinical profile viz.malaise ,fever weight loss, rash ,oral thrush, cough ,seizures ,lymphadenopathy ,diarrhea and the mean value of hemoglobin level, RBC indices viz. MCV, MCH, and MCHC, mean WBC count , mean platelet count ,mean platelet volume in HIV positive patients before and after 3 months of ART were as shown in following table .
- Overall prevalence of anemia was higher in Pre-ART participants as compared to post-ART 46% and 28 % respectively; this difference was statistically significant. Followed by thrombocytopenia (24% and 21%),leucopenia (8% and 9%) in pre-ART and post-ART participants ,respectively. This difference in platelets counts and TLC was not statistically significant.
- Table 2 shows that majority i.e 38% participants reported with malaise, followed by fever 27%, weight loss 27%, rash 14%, oral thrush 13%, cough 11%, seizure 2%, and only 1% participants reported with lymphadenopathy and 1% with diarrhea before taking ART. A significant change was seen following three months on ART in which only 15% of the individuals in the research experienced malaise, 9.0% experienced weight loss, 3.0% experienced fever, and just 1.0% experienced cough and rash. There were no case of lymphadenopathy, seizure episodes, diarrhea, or oral thrush among the subjects after 3 months of ART .
- Table 3 shows hemoglobin (hb) , mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH),mean corpuscular hemoglobin concentration (MCHC) increase significantly after 3 months of ART in HIV positive patients whereas the change in platelets counts , mean platelet volume(MPV) ,total leucocyte counts(TLC) was not statistically significant.

Table 1:- Distribution of study participants according to age,sex and route of transmission of HIV.

Basic Details	Mean ± SD ||Min-Max OR N (%)
Age (Years)	37.90 ± 11.66|| 18.00 - 74.00
Age	
18-30 Years	34 (34.0%)
31-40 Years	34 (34.0%)
41-50 Years	15 (15.0%)
51-60 Years	15 (15.0%)
61-70 Years	1 (1.0%)
71-80 Years	1 (1.0%)
Gender	
Male	57 (57.0%)
Female	43 (43.0%)
Route Of Transmission	
HS	35 (35.0%)
IVDU	25 (25.0%)
Needle Injury	22 (22.0%)
Unknown Source	17 (17.0%)
Mother To Child	1 (1.0%)

Basic Details	Mean ± SD ‖Min-Max OR N (%)

Table 2:-Comparative analysis of clinical profile of study participants before and after 3 months of ART.

Clinical profile	Baseline	After 3 months	McNemar test(x2)	p-value
Malaise	38(38%)	15(15%)	23.000	0.001
Fever	27(27%)	3(3%)	24.000	0.001
Weight loss	27(27%)	9(9%)	18.000	0.001
Rash	14(14%)	1(1%)	13.000	0.001
Oral thrush	13(13%)	0(0%)	13.000	0.001
Cough	11(11%)	1(1%)	8.333	0.004
Seizures	2(2%)	0(0%)	2.000	0.157
Lymphadenopathy	1(1%)	0(0%)	1.000	0.317
Diarrhea	1(1%)	0(0%)	1.000	0.317

Table 3:-Comparative analysis of Mean ±SD value of various hematological parameters in study participants before and after 3 months of ART.

Hematological parameters	Baseline (Mean±SD)	After 3 months (Mean±SD)	Mean change (Mean ±SD)	Wilcoxon test	p-value
Hemoglobin (gm/dl)	10.95 ± 2.38	12.41 ± 2.13	1.46 ± 2.93	1108.5	<0.001
MCV (fl)	84.97 ± 8.01	86.79 ± 9.37	1.82 ± 10.35	1795.0	0.012
MCH (pg)	27.93 ± 7.59	29.52 ± 8.04	1.59 ± 11.70	1294.0	<0.001
MCHC (g/dl)	32.13 ± 1.50	32.80 ± 2.01	0.67 ± 2.53	1485.5	0.005
TLC (10^3/µl)	8.07 ± 4.29	7.97 ± 5.82	-0.10 ± 6.86	2677.5	0.373
PLC (Lacs/µl)	2.10 ± 0.88	2.19 ± 1.03	0.09 ± 1.16	2265.5	0.572
MPV (fl)	11.20 ± 1.68	11.52 ± 1.87	0.32 ± 1.98	1769.5	0.151

Table 4:-Comparative analysis of Mean ±SD value of various parameter of liver function test in study participants before and after 3 months of ART.

LFT	Baseline Mean ± SD	After 3 months Mean ± SD	Mean change Mean ± SD	Wilcoxon test	p-value
Total Bilirubin (mg/dl)	0.70 ± 0.10	0.65 ± 0.38	-0.05 ± 0.38	2493.0	0.045
AST (IU)	34.33 ± 2.66	62.60 ± 135.63	28.27 ± 135.74	1952.5	0.068
ALT (IU)	35.14 ± 4.19	48.33 ± 59.17	13.19 ± 59.37	2415.5	0.708
ALP (IU)	95.84 ± 22.33	133.61 ± 107.14	37.77 ± 108.25	1758.0	0.008

Table :-5 Comparative analysis of Mean ±SD value of various parameter of renal function test of HIV positive patients before and after 3 months of ART.

RFT	Baseline Mean ± SD	After 3 months Mean ± SD	Mean change Mean ± SD	Wilcoxon test	p-value
Urea (mg/dL)	30.71 ± 6.30	29.47 ± 13.70	-1.24 ± 14.55	2685.5	0.192
Creatinine (mg/dL)	0.76 ± 0.20	0.88 ± 0.91	0.12 ± 0.94	1644.5	0.532

Discussion

Hematological parameters and clinical profile are important monitoring tools for assessing treatment and prognosis in HIV. The aim of our study was to evaluate the hematological parameters and clinical profile among HIV positive patients on antiretroviral therapy for at least 3 months and treatment-naïve patients and to do their comparative analysis.

Age and sex : In present study, the majority of participants 34% belonged to the age group of 18-30 years and 34% belonged to 31-40 years followed by 15.0% of the participants to 41-50 years and 15.0% of the participants to 51-60 years. Only 1.0% of the participants belonged to 61-70 years & 71-80 years. The mean age (years) was 37.90 ± 11.66. A similar study by Jagtap SB. showed that the majority of cases belonged to the age group of 31-40 years i.e 28%. Another studies by Vyas Y & Khan Y, showedthat 72.27% of the patients of age group 30 to 49 years, and 45% to the age group 31-40 years were affected respectively(22,23,24).In our study, males 57% were more common than females 43% and the male to female ratio was 57:43.

Most studies had shown similar trends where males were predominantly present in the studies in comparison to females (22,23,24). Causes of male predominance can be because women receive less attention and looked after in our society as compared to males leading to higher number of male candidates being brought for routine check ups and follow up. In contrast to this study by Girum T et al., showed that as compared to men, adult women are 1.62 times more likely to have HIV.A similar trend was shown by two other studies(25,26,27)**.**

Route of transmission

In present study,35.0% of the participants had HIV infection by heterosexual route of transmission, followed by 25.0% by intravenous drug use, 22.0% by needle injury, 17.0% by unknown source and only 1.0% of had route of transmission from mother to child. Most studies have shown a similar result: that heterosexuals route was the most common way that persons contract HIV(28,29).In contrast, to this study by Bhunu CP et al. showed that Intravenous drug use and tattooing remain two of the major routes of HIV/AIDS transmission among prisoners (30).

Clinical profile: Variation was observed in the presenting symptoms in present study majority 38% participants had malaise, followed by fever 27%, weight loss 27%, rash 14%, oral thrush 13%,cough 11%, seizure 2%, lymphadenopathy 1% and diarrhea 1%(baseline) whereas in contrast to our study, weight loss was seen as the most common symptoms in Shakira Vijay Savakar et al, Lodha et al., and Sharma et al. studies (56.36%, 81.3%, and 26% of patients respectively) (31,32,33).

Significant improvement was observed in the clinical profile of participants after 3 months of ART and after ART, 15% of participants had malaise, 9.0% of the participants had weight Loss, 3.0% of the participants had fever, 1.0% of the participants had cough, and 1.0% of participants had rash. No participant had diarrhea, oral thrush, lymphadenopathy, or a seizure episode. A similar effect of ART was seen in a study by Shakira Vijay Savakar et.al(31). In contrast to our study, despite the benefits of ART, HIV-infected children remain at risk for many common illnesses (opportunistic infections) as shown in study conducted by Mubiana- Mbewe M et al (34).

Hematological parameters : In the present study, the mean hemoglobin level of 12.41 ± 2.13 g/dl in ART experienced patients was significantly higher than the mean hemoglobin level of 10.95 ± 2.38 g/dl in ART- naïve patients with a p value of <0.001.This was probably due to an improvement in hemoglobin concentration after antiretroviral therapy. This was in concordance with a study conducted by Jasneet Kaur et al. with a mean hemoglobin value of 10.23±1.81 g/dl in ART experienced patients and 8.52±1.89 g/dl in ART- naïve patients. In contrast, to this study by Duguma N

et al. showed the prevalence of anemia, leukopenia, and thrombocytopenia before initiation of antiretroviral treatment was higher,although anemia and thrombocytopenia decreased correspondingly after initiation of treatment, leukopenia increased by 4%. CD4+ T-cellcount <200 cells/μL was the sole independent risk factor for anemia and leukopenia before highly active antiretroviral therapy, while stage IV disease, female sex, zidovudine, lamivudine, and nevirapine treatment,and intestinal parasite infection were predictors of anemia after
treatment initiation (35 36)**.**

RBC indices: In the present study, mean value of MCV is 86.79 ± 9.37 inpost ART participants and 84.97 ± 8.01 in pre ART participants, which is significantly higher when subjected to statistical analysis. This improvement in MCV in participants on ART is similar to the study by
Jasneet Kaur et al. with a mean MCV of 83.31±7.30 fl in treatment naïve HIV- reactive patients and 89.40±8.34 fl in patients on ART. Similarly, a study by Damtie S et al. also showed an increase in MCV after ART (87.17 ±3.51 fl vs. 100.67 ±4.9fl) (35,37).The mean value of MCH in our study was 29.52 ± 8.04 in post-ART participants and 29.52 ± 8.04 in pre ART participants, which is significantly higher when subjected to statistical analysis. This improvement in MCH in patients on ART is similar to study by JasneetKaur et al. and Damtie S et al. with mean MCH (27.52±2.30pg vs.28.98±1.81pg) and (28.75±3.19 pg vs. 34.04±4.15 pg) in patients pre-ART and post- ART respectively(35,37).The difference in MCHC before and after initiation of ART was statistically significant in present study. The mean value of MCHC was32.80 ± 2.01 in post- ART participants and 32.13 ± 1.50 in pre- ART participants. These observations were in concordance with the study byDamtie et al in which mean cell hemoglobin concentration (MCHC)(328.6±20.3 g/l vs. 338.9±18.3 g/l), was pre- and post- ART respectively,and opposite result was seen in the study by Jasneet Kaur et al. and by Parinitha et al.(35, 37,38)**.**The prevalence of leucopenia was reported to be 8% in the present study. It was slightly higher, i.e 9%, in post-ART participants as compared to 8% in pre-ART. In concordance with this leucopenia was observed to be more common in patients receiving antiretroviral therapy,
as in the study by Jasneet Kaur et al. & Damtie et.al. Leukocytosis was seen in 12% participants who were not on ART. It might be caused by anenhanced immune response to opportunistic infections. In the present study, the mean TLC ($10^3/\mu L$) (baseline) was 8.07 ± 4.29 & after 3 months the mean TLC ($10^3/\mu L$) was7.97 ± 5.82. This change in mean value was not statistically significant(35 37).In present study, prevalence of thrombocytopenia was 24%; the mean MPV (fl) (baseline) was 11.20 ± 1.68 in pre- ART and 21%, the mean(MPV=11.52±1.87) in post ART participants. Although there is a difference in the overall prevalence of thrombocytopenia and MPV in the comparative studies, But it was not statistically significant .In contrast toour study, studies by Jasneet Kaur et al. and Damtie et al.showed a statistically significant difference with the prevalence of thrombocytopenia was {21.87% in group A (pre-ART) vs. lower i.e.12.5% in group B (post-ART)} and 17.1% and 8.3% before and after 6months of HAART initiation, respectively(35,37).

Renal function test

In present study, the mean value of urea (mg/dl) (baseline) was 30.71 ±6.3 and creatinine (mg/dl) (baseline) was 0.76 ± 0.20, and after ART mean urea (mg/dl) was 29.47 ± 13.70 and mean creatinine (mg/dl) was0.88 ± 0.91. This change was not statistically significant (p = 0.192).Conversely study conducted by Fiseha T, Gebreweld A, and ChapondaM, Pirmohamed M. showed a significant impairment in renal function tests after ART (39, 40)**.**

Liver function test

All antiretroviral therapy medications have been shown to cause nephrotoxicity, which can range from acute kidney injury to chronic kidney disease, and hepatotoxicity, which can range from transaminitis to frank liver failure On the other hand, in the liver function test, the mean
total bilirubin in the present study after three months was lower. A rise in alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) was observed. But in this ALP changed significantly, although AST and ALT did not alter
significantly(40).

CONCLUSION

From this study it can be concluded that Anemia, leucopenia, and thrombocytopenia were found to be the most common hematologic abnormalities in HIV/AIDS patients. Clinical profile and hematological parameters (hemoglobin, MCV,MCH, MCHC) had statistically significant differences before and

after HAART initiation. Alterations in the hematological parameters are very frequent in both newly diagnosed treatment naïve HIV-reactive patients and those patients on antiretroviral
therapy. The present study highlights that antiretroviral therapy has the capability of reducing the prevalence of anemia, lymphopenia, thrombocytopenia, and other deranged
hematological parameters provided that the patient maintains proper adherence to the therapy and an appropriate dosage and drug regimen is selected by the clinician. Additionally, large-scale and longitudinal studies are recommended to strengthen and explore the problem in depth.

LIMITATION

Our study was a single center study with a limited sample size of 100. A large sample size would have provided more information regarding the HIV-positive patients.

Follow-up in our study was of 3 months. A longer follow-up period can help for be understanding the effect of ART on clinical and hematological parameters in HI positive patients.

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