Original Article

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A Comparative Study of ART Regimens and the Haematological Effect of ART in Treatment of PLHIV

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Abstract

Background: Haematological manifestations are the second commonest cause of morbidity and one of the common causes of mortality in HIV patients. In the course of ART administration, clinically significant haematologic abnormalities are common in persons with HIV infection. *Objectives*: To study the comparison of haematological parameters in subjects with different CD4 counts, to compare haematological findings of pre-ART group with ART group and two ART regimens namely ZLN and TLE. *Study Design*: Prospective observational study. *Material and Methods:* After obtaining informed consent, 225 PLHIV were clinically evaluated and subjected to CBC, PBS, ALC, ESR and CD4 count. *Statistical Analysis*: Data was expressed as percentage and mean ± standard deviation. Statistical tests used were chi-square test, Fisher's exact test and Mann-Whitney U test and Kolmogorov-smirnov analysis. *Results*: TLC ($p=0.02$), MCV($p=0.04$) and ALC ($p<0.0003$) were significantly raised while Hb, MCH, MCHC, PCV, ESR, CD4 count showed Non significant raised values in ART group in comparison to pre-ART group. MCH ($p=0.01$) and ALC ($p<0.001$) were significantly raised and Hb, MCHC, PCV, ESR, ALC showed Non significant raised values in PLHIV on TLE regimen when compared to ZLN regimen while TLC ($p=0.04$) was significantly raised in ZLN regimen. *Conclusion:* Most significant parameter was ALC in comparison between treated and untreated PLHIV groups and two ART regimens. TLE regimen fared better than ZLN regimen in treatment of PLHIV.

Keywords: Haematological; PLHIV; TLE; ZLN.

Introduction

The first AIDS case in India was detected in 1986 [1]; since then the spread of HIV in India has been diverse, epidemic being most extreme in the southern half of the country and in the far North-East [2].

HIV infection is associated often with a wide range of haematological abnormalities, including impaired haematopoiesis, immune mediated cytopenias and coagulopathies, particularly in the later part of the disease [3].

Though many studies have been conducted, in most of them, various aspects were addressed and the focus on the haematological manifestations was limited. Most of the available data is from the west, which might not be directly applicable to the Indian subcontinent. Hence, this study was conducted using one of the most easily available investigations (complete haemogram) in a medical college-based observational study of HIV-infected adults attending R.C.S.M. GMC, Kolhapur. Even after thorough research we were unable to find any study which compared haematological parameters between two common ART regimens (ZLN and TLE).

Material and Methods

HIV seropositive patients referred from ART centre of R.C.S.M Govt Medical College, Kolhapur for CBC to haematology department, central clinical laboratory (CCL) from March 2015 to March 2016 were included in this study, irrespective of their ART status. Written informed consent was obtained from all. Patients were excluded if they were less than 18 years of age or were pregnant or refused to become part of study.

After taking informed consent, detailed clinical history of every subject was recorded which included

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history of any opportunistic infection and treatment taken. Also, general and systemic examination was done. Haematological parameters (Hb, TLC, DLC, Platelet count, MCV, MCH, MCHC, HCT) were determined using the haematology auto analyzer CounCell-23 plus whereas the immunological parameter (CD4+ T cells) were assayed using the BD FACS Count system. Differential leucocyte count and patterns of anaemia were studied on peripheral blood smear stained with Leishman stain. ESR was carried out. Absolute lymphocyte count (ALC) was calculated as-

ALC (cells/cu.mm) = Differential lymphocytes (%) X TLC (cells/cu.mm)

The collected information was compiled in a predetermined proforma.

Anaemia was defined as haemoglobin <13g/dl (men) and <12g/dl (women), leucopenia as total leucocyte count <4000cells/µl, neutropenia as absolute neutrophil count <1000cells/µl, lymphopenia as absolute lymphocyte count <800cells/µl and thrombocytopenia as platelet count <150×10^3 cells/µl.

Ethics

The study was carried out after taking permission from the Institute's Ethical Committee and MSACS (Maharashtra State AIDS Control Society).

Statistics

Descriptive statistics were expressed as percentage and mean±SD. Statistical tests used were chi-square test, Fisher's exact test and Mann-Whitney U test. Kolmogorov-smirnov analysis was used to assess the linearity of the data. Pearson's rank order correlation was used to assess the correlation between two parameters. P<0.05 was treated as statistically significant. SPSS Vs. 16 (IBM Corp) ® and Microsoft excel (Microsoft corp. pvt. ltd. ™) were used to perform the statistical analysis.

Results

Total number of HIV seropositive patients presenting in CCL in the prescribed period was 356. The number of cases meeting the inclusion criteria was 235. 10 cases had insufficient data so they were excluded from the study. Hence, the sample size for study was 225. Majority of HIV positive patients in our study belonged to 30-40 years age group (36.4%, n=50) followed by 40-50 years (29.3%, n=82). Out of the 225 subjects, 123 (54.6%) were males and 102 (45.3%) were females.

Most of the patients in our study i.e. 205 (91%) were receiving ART while 20 (9%) patients were not receiving ART(i.e. Pre-ART). Out of these 205 subjects, 124 (55.11%) subjects were on ZLN therapy and 81(16.44%) subjects on TLE therapy.

In our study, maximum patients presented with symptom of weight loss 156 (69.3%) followed by 80 (35.5%) patients with fever, 53 (23.5%) patients with cough and 12 (5.33%) patients with abdominal pain. Few of the patients presented with multiple symptoms mentioned above. Predominant sign was pallor present in 34 (15.1%) subjects followed by other signs such as lymphadenopathy in 15 (6.7%), icterus in 4 (1.78%), clubbing in 3 (1.33%) and cyanosis in 1

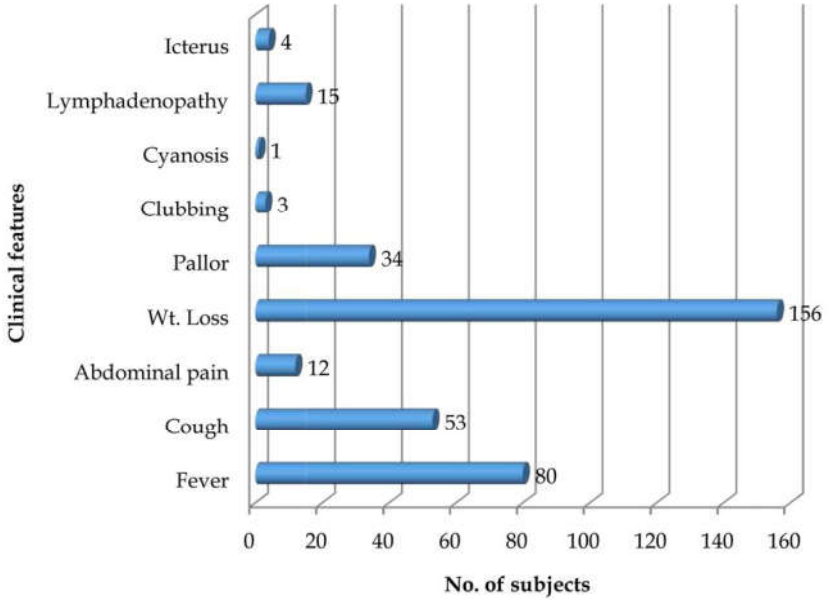

Fig. 1: Clinical features in study subjects

Table 1: Comparison of haematological parameters in Pre-ART (HIV positive patients not on ART) subjects and HIV positive subjects on ART

Hematological parameters	Pre-ART (Mean ± SD)	ART (Mean ± SD)	P value
TLC (cells/cu.mm.)	4950±1633.8	5700±2048.7	0.02
Differential neutrophil%	64±8.1	61±8.2	0.43
Differential lymphocyte%	17±8.5	18±10.6	0.5
Differential monocyte%	2±0.4	2.0±0.8	0.61
Differential eosinophil%	1±0.2	1±0.2	1
Differential basophil%	1±0.8	1±0.5	0.8
Hb (g%)	10.25±1.9	12.1±2.2	0.08
MCV(fl)	79.5±11.8	91.9±13	0.04
MCH(pg)	26.35±6.2	30±6.7	0.07
MCHC(g/dl)	31.95±3.8	34.1±3.6	0.9
PCV (%)	30.5±5.2	33.4±4.9	0.3
ESR(mm/hr)	16±4.2	14±4.4	0.4
CD4 count(cells/cu.mm)	193±341.0	210±283.1	0.09
ALC (cells/cu.mm)	1089±527.8	1225±586.8	0.0003
Platelet count(cells/cu.mm)	2.565±0.8	2.6±0.6	0.5

Table 2: Comparison of haematological parameters in study subjects on two different ART regimens (ZLN and TLE)

Haematological parameters	TLE	ZLN	P value
TLC (cells/cu.mm.)	5600±2400.1	5700±1779.7	0.04
Differential neutrophil %	60.1±8.9	62.0±7.6	0.5
Differential lymphocyte %	18±10.6	18.5±10.7	0.9
Differential monocyte %	2.0±0.7	2.0±0.8	0.5
Differential eosinophil %	1.0±0.2	1.0±0.3	0.5
Differential basophil %	1.0±0.6	1.0±0.5	0.5
Hb (g%)	12.4±2.3	11.3±2.1	0.4
MCV (fl)	96.8±13.3	91.3±12.9	0.06
MCH (pg)	34.5±6.7	28.2±6.6	0.01
MCHC (g/dl)	35.9±3.9	33.4±3.4	0.08
PCV (%)	33.4±4.3	33.0±5.3	0.6
ESR (mm/hr)	15.0±4.7	14.0±4.3	0.09
CD4 count (cells/cu.mm)	246.5±250.3	194±303.1	<0.001
ALC (cells/cu.mm)	1296±564.8	1156.0±602.9	0.08
Platelet count (cells/cu.mm)	2.5±0.6	2.6±0.6	0.5

(0.44%) subject (Figure 1).

Table 1 shows comparison of haematological parameters in Pre-ART (HIV positive patients not on ART) subjects and HIV positive subjects on ART. Table 2 shows comparison of haematological parameters in study subjects on two different ART regimens (ZLN and TLE).

Discussion

Haematological abnormalities may occur as a result of HIV infection itself, as sequelae of HIV-related opportunistic infections or malignancies or as a consequence of therapies used for HIV infection and associated conditions [4]. We evaluated 225 consecutive HIV seropositive patients who presented at Central Clinical Laboratory, RCSM GMC, Kolhapur, irrespective of their ART status. We also compared the final haematological diagnosis of patients on ART with Pre-ART group and the same between ZLN treatment group and TLE treatment group.

Availability of free antiretroviral drugs to HIV infected individuals has provided a new lease of life to HIV positive patients. Treatment of HIV infected patients with currently available highly active anti-retroviral therapy (HAART) drugs is successful in reducing the burden of disease but it's associated with various side effects [5].

The first line regimen of antiretroviral therapy (ART) as per NACO guidelines includes [5]-

-ZLN (Zidovudine [300mg] + Lamivudine [150mg] + Nevirapine [200mg])

-TLE (Tenofovir [300mg] + Lamivudine [150mg] + Efavirenz [600mg])

- ZLE (Zidovudine [300mg] + Lamivudine [150mg] + Efavirenz [600mg])

- TLN (Tenofovir [300mg] + Lamivudine [150mg] + Nevirapine [200mg])

205 patients in our study were on ART while 20 were pre-ART, this disparity can be due to different rates of patients coming to Haematology department, CCL for blood investigations. Pre-ART patients are not generally advised CBC by ART centre of our hospital while patients on ART undergo routine CBC monitoring.

Most of the HIV positive patients were symptomatic and presented with weight loss 156 (69.3%), fever 80 (35.5%), cough 53 (23.5%) and abdominal pain 12 (5.33%) (Figure 1). Dikshit B et al had observed that 82.5% patients in his study were symptomatic, out of them 54.5% complained of fever and 10.3% had loss of weight [6].

The predominant sign present in subjects of our study was pallor 34 (15.1%) followed by lymphadenopathy 15 (6.7%), icterus 4 (1.78%), clubbing 3 (1.33%) and cyanosis 1 (0.44%) (Figure 1).

Haematological abnormalities are among the most common complications of HIV which involves all lineages of blood cells. Results from the study showed that HIV infection affect haematological indices of patients regardless of age and sex.

Comparison of Haematological Parameters between Pre-Art Subjects and Subjects on ART

In the course of ART administration, clinically significant haematologic abnormalities maybe common in persons with HIV infection. Impaired haematopoiesis, immune-mediated cytopenias and altered coagulation mechanisms have all been described in HIV-infected individuals. Abnormalities may occur in individuals as a result of the following actions; HIV infection, sequel of HIV-related opportunistic infections, malignancies and consequence of therapies used for HIV infection and associated conditions [7].

In our study findings a significant variation was observed in some of the haematological parameters examined in HIV positive subjects on anti-retroviral (ART) treatment. The total WBC count shows a significant increase from pre-ART to ART. The significant increase in mean values from pre-ART to patients on ART observed in absolute lymphocyte ($p=0.003$) and total WBC ($p=0.02$) may indicate suppressive activity of the antiretroviral drug on the virus with the resultant decrease in leucopenia and lymphocytopenia (Table 1). Leucopenia and lymphocytopenia are the hallmarks of HIV infection and is thought to be mediated by infection of the virus with subsequent killing of CD4+ T cells. It can also be caused by certain medications such as ART and certain infections thereby decreasing TLC and ALC such as in studies by Enawgaw et al and Ibeh et al [7,8,9]. The significant increase found in the TLC from pre-ART to ART follows the pattern already reported by Amegor OF et al [10].

The result showed an increased mean PCV from pre-ART to patients on ART, the data tend to have similar progression with the values obtained from Haemoglobin (Table 1).

This suggests an effective therapeutic effect of the drug since a decreased PCV indicates anaemia. Mean haemoglobin increases in patients who receive ART thus reversing HIV associated anaemia which is consistent with studies by Enawgaw B et al ($p<0.001$), Ibeh BO et al and Amegor OF et al and [8-10]. The mean values of MCV ($p=0.04$), MCH, MCHC were all increased in ART patients compared to patients not on ART, similar trend was present in study by Enawgaw B et al wherein all the above parameters were raised significantly ($p <0.001$) [8].

The reduction of ESR(16 ± 4.2 to 14 ± 4.4mm/hr) attest to the reduced anaemic condition found in the HIV subjects on anti-retroviral therapy. ESR decreased when compared with the pre-ART, ESR often may rise significantly in individuals due to infection or medication and merely reflect the anaemic condition seen in these subjects (Table 1) [11]. Ibeh BO et al have reported reduced mean ESR in HIV patients on ART (40.33mm/hr) compared to pre-ART (41.46mm/hr) [9].

A reduction in mean differential granulocyte count was observed between the pre-ART and ART (Table 1). It is of note that abnormal granulopoiesis and anti-granulocyte antibodies have been noted and described in HIV infected patients [12]. This is believed to contribute to the observed increase in neutropenia. The low granulocyte count seen in the ART patients may reflect the action of anti-retroviral drugs on HIV infection or associated conditions [13].

The incidence of comparative neutropenia in treated patients is consistent with other report by Ibeh BO et al which have also shown a high incidence of granulocytopenia particularly in patients with more profound immunodeficiency [9].

A reduced platelet count may indicate disease progression and may sometimes be associated with abnormal bleeding. Thrombocytopenia may result due to immune system malfunction [14]. The result here showed increase in platelet count from the pre-ART to ART (Table 1) which is consistent with studies by Enawgaw B et al and Ibeh BO et al [8,9]. This indicates a reduced incidence of thrombocytopenia in the HIV positive ART subjects. This action maybe adduced to the activity of the administered anti-retroviral therapy.

HIV patients accessing ART have been reported by Enawgaw et al (p=0.038) and Amegor OF et al to have an increased CD 4 cell count such a trend was also observed in our study [8,10]. Further investigation is needed to ascertain these haematological findings since in our study just 20 patients were pre-ART compared to 205 ART patients hence its limitation.

Comparison of Haematological Parameters between Subjects on ZLN and TLE Antiretroviral Therapy

In low resource settings, fixed-dose combinations of nucleoside reverse transcriptase inhibitors (NRTIs) such as Tenofovir and Lamivudine; and non-nucleoside reverse transcriptase inhibitors (NNRTIs) such as Efavirenz, are commonly used as antiretroviral therapy (ART) for HIV-infected patients in order to increase adherence to lifelong treatment.

Our study showed significantly decreased mean MCH (p=0.01) and CD4 count (p<0.001) values in patients on ZLN as compared to patients on TLE though mean TLC was significantly higher (p=0.04) in patients on ZLN than patients on TLE anti-retroviral therapy (Table 2).

In a study conducted by Krishnan CR et al, the two ART regimens showed sustained improvement in the CD4 count but TLE (Mean CD4- 514.43 cells/cu.mm) showed a slightly more increase in CD4 than ZLN (Mean CD4- 471.42 cells/cu.mm). The incidence of adverse effects (i.e. anaemia) was more in ZLN regimen compared to TLE. This study showed that TLE is safer than ZLN and the efficacy is relatively similar in both the regimens [15]. CD4 count comparison of ZLN and TLE regimens done by Hemasri M et al showed there is no significant difference between the two regimens, both had equal efficacy profile, during treatment with ZLN regimen mean CD4 was 358.43 while during TLE regimen mean CD4 was 322.95 and thus concluded that even though the combination of ZLN is very efficacious as a anti retroviral drug regimen, but TLE should be preferred by the physicians in Govt. general hospital [16].

However, even after thorough research, studies on haematological parameters observed during ZLN and TLE treatment were not found thus, there is a lacuna in literature.

HAART recovers neutropenia, lymphopenia, thrombocytopenia, anaemia. Relatively less changes in haemoglobin, PCV and platelet count in HIV seropositive patient could be an indication of low toxicity in patients on HAART [17]. A possibility of coexisting iron deficiency anaemia and megaloblastic anaemia couldn't be ruled out as serum ferritin and serum Vit B12/Folic acid level respectively wasn't done.

Anaemia, neutropenia, lymphocytopenia and thrombocytopenia are reversible by HAART so HAART should be started earliest after diagnosis of HIV infection and determining the CD4 count. ART drug toxicities are common findings so accordingly the treatment should be modified. Hence, routine monitoring of haematological parameters in patients with HIV/AIDS is recommended, to detect the abnormalities at the earliest, find the aetiology and treat appropriately. These measures will reduce the morbidity and mortality.

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