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Evaluation of Hematological Parameters in HIV Reactive Patients on Antiretroviral Therapy and Treatment Naïve Patients: A Comparative Study.

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ABSTRACT

Background: Hematological parameters are important monitoring tools for assessing treatment and prognosis in HIV. The use of antiretroviral drugs could positively or negatively affect these parameters, depending on the choice of combination used. The primary objective of the present study was to evaluate the hematological parameters among HIV reactive patients on antiretroviral therapy for at least 6 months and treatment naïve patients and their comparative analysis. The secondary objective was to correlate the hematological parameters of all the patients with CD4 cell count. **Methods:** This analytical, prospective, cross-sectional study was conducted for a period of 18 months on 200 HIV reactive patients enrolled as per selection criteria in the department of Pathology, Rajindra hospital Patiala .Hematological parameters viz. Hemoglobin concentration, ESR, hematocrit (PCV), RBC indices like MCV, MCH, MCHC & Red cell distribution width, TLC, DLC, ANC, ALC, platelet count, complete PBF examination for type of anemia and absolute CD4 cell count were done and comparatively analyzed amongst the two groups. **Results:** The prevalence of anemia, leucopenia and thrombocytopenia was relatively more in HIV infected treatment naïve patients as compared to those on ART and there was a positive correlation between derangements of these parameters with fall in CD4 cell count. **Conclusion:** The present study highlights that antiretroviral therapy has the capability of reducing the prevalence of anemia, lymphopenia, thrombocytopenia, and other deranged hematological parameters; and most importantly improving CD4 cell counts.

Keywords: Hematological parameters, HIV reactive patients on ART, treatment naïve patients, CD4 cell count.

INTRODUCTION

Hematological complications involving all the hematopoietic elements are probably the most frequently encountered complications of AIDS.[1] Many of these complications have been shown to occur with increasing frequency as HIV infection progresses and a variety of mechanisms appear to play a role in their evolution; such as direct and indirect effects of HIV infection, to the myelosuppressive drugs used in opportunistic infections ,and of drugs used as a part of antiretroviral therapy.[2]

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Anemia

Anemia in HIV-infected patients is likely to be multifactorial. Inflammatory cytokines play a central role in the pathogenesis of anemia. TNF, IL-1 and interferon gamma have all been shown to inhibit erythropoiesis in vitro.[3] TNF levels have been shown to be consistently elevated in HIV infection and correlate with viral load.[4]

The Effect of HAART on Anemia

Nucleoside analogs other than ZDV are not associated with anemia. Zidovudine is commonly associated with marrow toxicity, particularly with long term administration; but by use of lower doses of it in combination with other non-myelosuppressive antiretroviral drugs such as protease inhibitors, has significantly decreased the frequency of anemia.[5]

Leucopenia

Leucopenia generally correlates with the severity of a clinical syndrome like anemia.[6] Due to the lack of laboratory technologies in resource-limited countries, WHO guidelines suggest the use of simple tests such as hemoglobin (Hb) <12 g/dl and total lymphocyte counts (TLC) <1,200/cumm as indicators for initiation of antiretroviral therapy (ART).[7]

Thrombocytopenia

The mechanism of thrombocytopenia in HIV infection appears to involve increased platelet destruction and ineffective platelet production. Most reports indicate that there is significant platelet sequestration and destruction in the spleen in HIV-associated thrombocytopenia.[5]

Effect of HIV infection on RBC indices

HIV infection affects hematological indices like MCV, MCH and MCHC of patients regardless of age, sex and ART.[8]

Henceforth, it is concluded that hematological parameters are important monitoring tools for assessing treatment and prognosis in HIV. The use of antiretroviral drugs could positively or negatively affect these parameters, depending on the choice of combination used. Despite the presence of few reports on the hematological parameters of HIV reactive patients, comparative studies between HAART naïve and patients on HAART is scarce, and that is why, we planned the present study. The hematological parameters will also be correlated with CD4 cell count so that they may be used for monitoring at places where facility to assess CD4 cell count is not available.

MATERIALS AND METHODS

Study Design

This analytical, prospective, cross-sectional study was conducted for a period of 18 months. Two hundred HIV reactive patients were enrolled in the study amongst total number of individuals who visited ART Centre or referred from other departments of Rajindra hospital Patiala to the hematology section for complete haemogram and CD4 cell count, on the basis of inclusion and exclusion criteria for selection.

All HIV infected patients > 12 years of age of either sex and who were willing to participate in the study were included. Patients with age < 12 years, pregnant females, patients with previously known or congenital haematological disorder, and those with intercurrent infection unrelated to HIV with significant effect on haematological profile, were excluded from the selection criteria.

The patients were subdivided into two groups:

Group A: Newly diagnosed Treatment Naïve HIV reactive patients.

Group B: HIV reactive patients on ART for atleast 6 months and above.

Study Sequence

Detailed history from patients regarding route of transmission, occupation, clinical features, any other past chronic disease, was obtained. Hematological parameters viz. Hemoglobin concentration, ESR, hematocrit (PCV), RBC indices like MCV, MCH, MCHC & Red cell distribution width, TLC, DLC, ANC, ALC, platelet count, complete PBF examination for type of anemia and absolute CD4 cell count were done and comparatively analyzed amongst the two groups.

Procedure

Haematological parameters were analysed in haematology auto analyser Sysmex XP-100 which analyses using three detector blocks. Differential leucocyte count and type of anemia was studied on peripheral smear stained with Leishmann stain. Erythrocyte sedimentation rate (ESR) was measured by Westergren method.

CD4 lymphocyte count was done in BD FACS Count flow cytometer, The Centers for Disease Control and Prevention (CDC) criteria were used to classify the study population into three categories based on CD4 counts: stage 1 (CD4>500 cells/µL), stage 2 (CD4 between 200 and 499 cells/µL) and stage 3(CD4<200 cells/µL).

Hematological parameters were then correlated with the CD4 cell counts being divided into three groups:

i) Less than 200,

ii) From 200 to 500,

iii) More than 500 cells.

Statistical tests used included mean, standard deviation, chi-square test (x2), and t test. The data was entered in a Microsoft Office Excel worksheet and statistical analysis was done using SPSS version 20.2. Descriptive statistics were applied; p value less than 0.05 was considered statistically significant.

RESULTS

- There were 96 patients in Group a, distributed among the age group of 17-67 years and consisted of 74 males and 22 females.
- There were 104 cases in Group B, distributed among the age group of 19-67 years and consisted of 69 males and 35 females.
- Majority of patients were in the range of 30-45 years with a mean age of 38.47 years in group A and mean age of 39.01 years in group B. There wasn't any statistical difference between mean ages in both the groups.
- Males comprised 71.5% and females comprised 28.5% of the total 200 patients enrolled in this study with a male to female ratio of 2.5:1.
- There were 74 males and 22 females in group A out of total 96 patients with a ratio of 3.4:1. There were 69 males and 35 females in group B out of total 104 patients with a ratio of 2:1.
- The mean value of hemoglobin level, RBC indices viz. MCV, MCH, and MCHC , PCV, RDW, mean WBC count, mean value of ANC, ALC, CD4 cell count , mean platelet count, mean ESR, in both the groups were as shown in following table:

  The overall prevalence of anemia was higher in group A i.e. 89.58% as compared to 65.38% in group B, and the difference was statistically significant. The prevalence of moderate anemia and severe anemia in group A was 67.77% and 12.5%

respectively, and in group B was lower i.e. 18.26% and 6.73% respectively; and this difference was statistically significant.

Normocytic normochromic anemia was the most common type of anemia in both the groups. It was followed by microcytic hypochromic anemia and dimorphic anemia in group A; whereas it was followed by macrocytic anemia, microcytic hypochromic and dimorphic anemia in group B in decreasing order of prevalence.

There were 79.80% patients in group B with normal WBC count and 70.83% in group A. The prevalence of neutropenia was almost similar in both the groups i.e. 11.46% in group A and 14.42% in group B. The prevalence of lymphopenia was higher in group A (13.54%) as compared to that in group B (2.88%) and this difference was statistically significant.

The prevalence of thrombocytopenia in group A (21.88%) was significantly more than that in group B (12.5%) with a p value <0.05.

When correlating hematological parameters with CD4 cell count in both the groups, there was a decline in the prevalence of anemia with increasing CD4 cell counts, i.e. a statistically significant correlation was found, with p value of 0.001; as shown in following table.

Table 1: Comparative Analysis of Mean±SD Values of Various Hematological Parameters in Both Groups.

Parameters	Group A	Group B	t-test	p value
Hb.(g/dl)	8.52±1.89	10.23±1.81	6.50	0.001
MCV(fl)	83.31±7.30	89.14±8.34	3.84	0.001
MCH(pg)	27.52±2.30	28.98±1.81	4.97	0.001
MCHC(g/dl)	31.77±3.72	32.62±3.58	1.63	0.104
PCV (%)	29.83±4.20	31.98±3.59	3.88	0.001
RDW (%)	15.67±2.59	14.56±1.70	3.57	0.001
TLC(cells/µl)	5544.06±2537.68	5046.92±1570.68	2.18	0.045
ANC(cells/µl)	3398.75±1704.07	2920.79±1091.98	2.38	0.021
ALC(cells/µl)	2005.41±1041.15	2040.89±618.09	0.30	0.772
Platelet Count (cells/µl)	195570.83±68898.94	225245.67±76453.49	2.89	0.004
ESR (mm in 1 hr)	46.91±17.38	36.67±16.01	4.32	0.001
CD4 Count (cells/µl)	176.65±82.06	428.61±126.66	16.82	0.001
Neutrophil%	60.78±8.68	56.87±7.37	2.76	0.006
Lymphocyte%	37.18±8.21	41.20±7.12	3.01	0.003

Table 2. Correlation of different grades of anemia with CD4 cell count.

	CD4 <200	CD4 200-500	CD4 >500	Total	X^2	p value
Mild Anemia	6	39	6	51	22.76	0.001
Moderate Anemia	46	34	4	84	10.53	0.001
Severe Anemia	11	8	0	19	6.13	0.013
Normal Hb.	3	27	16	46	6.00	0.012
Total	66	108	26	200	8.96	0.001

Table 3: Correlation of TLC with CD4 cell count.

	CD4<200	CD4 200-500	CD4>500	Total	X^2	p value
Normal TLC	39	87	25	161	13.07	0.001
Leucopenia	25	18	1	44	4.70	0.030
Leucocytosis	2	3	0	5	2.50	0.114
Total	66	108	26	200	8.77	0.001

Table 4: Correlation of platelet count with CD4 count.

	CD4< 200	CD4 200-500	CD4 >500	Total	X2	p value
Platelet Count n.	49	91	23	163	9.38	0.001
Thrombocytopenia	17	16	1	34	5.02	0.025
Thrombocytosis	0	1	2	3	1.33	0.248
Total	66	108	26	200	8.96	0.001

There was a statistically significant correlation between CD4 count and total leucocyte count. Leucopenia was found only in patients with CD4 count below 500.

There was a decline in thrombocytopenia with improvement in CD4 count showing statistically significant correlation.

DISCUSSION

Hematological parameters are important monitoring tools for assessing treatment and prognosis in HIV. The primary objective of the present study was to evaluate the hematological parameters among HIV reactive patients on antiretroviral therapy for atleast 6 months and treatment naïve patients and their comparative analysis. The secondary objective was to correlate the hematological parameters of all the patients with CD4 cell count.

Age and sex distribution

In the present study, majority of patients were in the age range of 30-45 years and mean age was 38.47±11.85 years in group A and 39.01±10.00 years in group B. There was male preponderance with male to female ratio of 3.4:1 in group A, and 2:1 in group B.

These findings were in concordance with study conducted by Parinitha et al,[9] study by Kotwal et al and that conducted by Rahman et al on 204 HIV infected patients on ART in Bangladesh.[10,11]

Evaluation of Hematological Parameters

RBC Parameters

In the present study, the mean hemoglobin level of 10.23±1.81g/dl in ART experienced patients was significantly higher than the mean hemoglobin level of 8.52±1.89g/dl in ART naïve patients with a p value of 0.001. This was probably due to improvement of hemoglobin concentration after antiretroviral therapy.

This was in concordance with study conducted by Denue et al,[12] with mean hemoglobin value of 11.89±1.61g/dl in ART experienced patients and mean hemoglobin of 9.76±2.82g/dl in ART naïve patients.

RBC indices: Mean value of MCV (mean corpuscular volume) in the present study was 83.31±7.30fl in treatment naïve HIV reactive patients and 89.40±8.34fl in patients on ART which was significantly higher (p value: 0.001). This observation was quite similar to study by Parinitha et al and Tripathi et al.[9,13]

The higher MCV found in group B in our study was similar to a study conducted by Enawgaw et al.[14]

Mean value of MCH in our study was 27.52±2.30pg in group A and it was 28.98±1.81pg in group B, which is significantly higher when subjected to statistical analysis. This improvement in MCH in patients on ART is similar to study by Enawgaw et al with mean MCH of 28.41±3.7 pg in group A and 32.52 ±5.2pg in group B.[14]

The difference in MCHC before and after initiation of ART was not statistically significant in our study. The mean value of MCHC was 31.77±3.72 g/dl in group A and 32.62± 3.58g/dl in group B.

These observations are in concordance with study by Parinitha et al and that conducted by Tripathi et al.[9,13]

Mean hematocrit in our study was 29.83±4.20% in group A and 31.98±3.59% in group B. Similarly, Tripathi et al reported mean hematocrit of 27.36% and it was found to be 31.31% in a study by Parinitha et al.[9,13]

Red cell distribution width: In the present study, Mean value of RDW-CV was found to be 15.67±2.59% in group A and 14.56±1.70% in Group B, which was in concordance to study conducted by Enawgaw et al and Parinitha et al in treatment naïve HIV reactive patients.[9,14]

Table 5: Comparing prevalence of anemia before and after ART with other studies.

Authors	Anemia	
	Group A	Group B
R Omoregie et al[15]	69.17%	51.15%
Mathews et al[16]	45.3%	35.64%
Enawgaw et al[14]	29.7%	11.7%
R Thulasi et al[17]	82%	74%
Present study	89.5%	65.38%

In the present study, 77 % patients were found to be anemic, with anemic population being more in treatment naïve group i.e. 89.58% and less in patients after 6-12 months of ART i.e. 65.38%. This probably indicates improvement in hemoglobin after ART.

This observation was in concordance with study conducted by Omoregie et al Mathews et al and Thulasi et al,[15-17] the prevalence of anemia in their studies was as shown in above table.

In the present study, normocytic normochromic type of anemia was the most common type of anemia in both the groups, followed by microcytic hypochromic anemia, then dimorphic anemia and finally macrocytic anemia being least common in group A i.e. treatment naïve HIV reactive patients whereas in HIV reactive patients on ART, normocytic normochromic anemia was followed by macrocytic anemia, and then similar incidence of dimorphic and microcytic hypochromic anemia.

These findings were similar to study done by Tripathi et al and Parinitha et al.[9,13]

WBC Parameters

In the present study, WBC count was found to be normal in 75% patients, similar to as found by Patwardhan et al i.e. in 75.6% patients and in 70.4% patients by Parinitha et al.[9,18]

Table 6: Comparing prevalence of leucopenia with other studies.

Authors	Leucopenia	
	Group A	Group B
Mathews et al[16]	8.14%	3.96%
Enawgaw et al[14]	16.6%	35.9%
Thulasi et al[17]	14%	25%
Present study	23.95%	20.19%

The prevalence of leucopenia was reported to be 22% in the present study. It was slightly higher i.e. 23.9% in group A as compared to 20.2 % in group B. In concordance to this, prevalence of leucopenia was found to be higher in group A i.e. 8.14% in the study by Mathews et al, and 3.96% in group B.[16]

In contrast to this, in a study by Enawgaw et al, and in a study by Thulasi et al,[14,17] prevalence of leucopenia was found to be slightly more in patients with ART. The difference might be due to different study population and different regime used for therapy.

Leukocytosis was found to be 2.5% and all the cases were reported in HIV reactive patients not on ART. It might be caused by enhanced immune response to

opportunistic infections. This finding was similar to study by Mathews et al.[16]

In the present study, mean TLC count was higher in treatment naïve patients (5544.06±2537.68 cells/µl) and significantly lower value was found in those patients on ART since 6 to 12 months (5046.92±1570.68 cells/µl). This was probably due to the effect of Zidovudine and Tenofovir in lowering the neutrophil count. That is why, although not statistically significant, prevalence of neutropenia was found to be 11.4% in group A and slightly more i.e. 14.42% in group B. This was in concordance with study done by Enawgaw et al.[14]

In the present study, prevalence of lymphopenia was reported to be 32.3% in group A and 10.7% in group B. This was probably due to improvement in lymphocyte count by antiretroviral drugs.

In concordance to this finding, in a study by Tripathi et al,[13] lymphopenia was seen in 21.1% of non-AIDS (HIV infected patients with CD4 more than 200) and 27.7% of AIDS (HIV infected patients with CD4 count less than 200).

Platelet Count

Table 7: Comparing prevalence of thrombocytopenia with various studies.

Authors	Thrombocytopenia	
	Group A	Group B
Mathews et al[16]	4.65%	2.95%
Enawgaw et al[14]	9%	4.1%
Thulasi et al[17]	9%	3%
Present study	21.87%	12.5%

Platelet count was normal in 81.5% patients i.e. 163 cases out of 200 had normal platelet count. Prevalence of thrombocytopenia was 21.87% in group A and lower i.e.12.5% in group B. This difference was found to be statistically significant.

Although there is difference in overall prevalence of thrombocytopenia in the comparative studies, but the improvement in platelet count after ART was similar to studies conducted by Mathews et al, Enawgaw et al and Thulasi et al.[14,16,17]

Correlation of prevalence of anemia, leucopenia and thrombocytopenia with CD4 cell count:

There was a significant correlation in the percentage of anemic patients and CD4 cell count. Moderate and severe anemia was more prevalent in patients with CD4 count less than 200 and those with CD4 count between 200-500, as compared to in patients with CD4 count more than 500. Prevalence of anemia increased as the CD4 count progressively decreased.

There was statistically significant correlation between WBC count and CD4 cell count. Almost 100% of patients with CD4 count above 500 had normal WBC count whereas normal WBC was found in 80.55% in patients with CD4 count between 200-500, and 59% in patients with counts less than 200.

There is a significant correlation in platelet count and CD4 count. Incidence of thrombocytopenia was found more in patients with lower CD4 counts than those with higher CD4 cell counts.

A study by Rahman et al also concluded a significant increase in the prevalence of anaemia, leucopenia,[11] lymphopenia and thrombocytopenia with decreasing CD4 cell count.

CONCLUSION

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; and most importantly improving CD4 cell counts; provided that the patient maintains proper adherence to the therapy and appropriate dosage and drug regime is selected by the clinician.

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