

Original Research Article

Peripheral haematological manifestations in HIV infection and its relation to CD4 count

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ABSTRACT

Background: HIV infection is associated with a wide range of haematological abnormalities, which are amongst its most common complications. This study aims at discerning the peripheral haematological abnormalities associated with HIV infection and to correlate them with CD₄ cell count.

Methods: An observational cross-sectional study was conducted from March 2017 till August 2018. 109 patients in 15 years or more age group who were HIV positive by the NACO guidelines were included. Complete hemogram, serum iron studies, serum folate and vitamin B12 levels, and flowcytometric CD₄ cell count analysis were done for all the patients. Various haematological parameters were compared between the patients with CD₄ cell counts <200/ μ l (n=52) to those with counts >200/ μ l (n=57). By using student t-test, the p-value was calculated for various parameters.

Results: Anaemia (58.7%), leucopenia (27.5%) and thrombocytopenia (17.4%) were seen with anaemia being the most common abnormality. Normocytic normochromic anaemia (65.6%) was the predominant type of anaemia. Overall analysis showed a statistically significant difference between two groups in haemoglobin concentration, RBC indices, serum ferritin values and absolute lymphocyte count; with p-value <0.05.

Conclusions: The diagnosis and treatment of haematological disorders are essential in medical care of the HIV-infected patients. Thus, in resource limited setups, where CD₄ count analysis is not possible, haematological abnormalities can be used as tools for monitoring HIV positive individuals and can aid in the treatment of the patients.

Keywords: Anaemia, CD₄ count, HIV, Haematological abnormalities, Haemoglobin

INTRODUCTION

HIV continues to be a serious health issue in most parts of the world. With a global prevalence of about 0.8% among adults, an estimated 36.9 million people were living with HIV, by the end of 2017. Vast majority of the cases are in developing countries with limited health care resources.¹

HIV infection is associated with a wide range of haematological abnormalities, which are amongst the

most common complications of HIV and are seen throughout the course of the infection. These abnormalities may be anaemia, leucocyte disorders, thrombocytopenia or pancytopenia.

Peripheral blood changes usually encountered in HIV infected individuals and disease consociated with HIV may reflect disease elsewhere in the body, may result from treatment for that disease, may reflect an attempt to attack the HIV itself, or may seem to be isolated haematological disorders.²

The CD4 lymphocyte is the primary target of HIV infection that leads to progressive deterioration of cellular functions, characterized by a gradual decline in peripheral blood CD4 lymphocyte levels.³ Reduction in absolute number of CD4 T cells occurs as one of the earliest immunologic abnormalities of HIV infection and is the most important prognostic indicator for risk of developing opportunistic infections.⁴ The present recommendation for testing CD4 cell count is every 6 months. However, the test is costly and uses sophisticated equipment and moreover, is not readily available in the peripheral setup.

Though there have been a few studies in India for evaluating the role of haematological variables as an alternative marker for CD4 counts, limited data is available in the Indian resource limited setting, specially from Central India regarding the correlation between haematological parameters and CD4 counts. This study aims at discerning the haematological abnormalities associated with HIV infection, to correlate them with the CD4 cell count and to foreground these manifestations with disease progression.

METHODS

The present study is a cross-sectional observational study done at Netaji Subhash Chandra Bose Medical College, Jabalpur between March 2017 to August 2018. The study was conducted in 109 HIV positive patients after obtaining permission from the Institutional Ethics Committee.

Sample size calculation and subject selection

Sample size was determined by using the formula z^2pq/d^2 considering the prevalence rate of HIV in the area. 109 patients satisfying strict inclusion and exclusion criteria were enrolled for the study after getting their written informed consent. Clinical history was elicited from each of the patient or guardians and general and systemic examination was carried out with emphasis on signs suggesting haematological system involvement. Clinical examination findings along with their socio-demographic data were recorded in a structured schedule case record proforma for every patient.

Procedure

Approximately 10 ml blood sample was drawn by venipuncture from each of the patient under strict aseptic precautions and was sent for carrying out the required laboratory investigations, using the EDTA and clot activator (Plain) vacutainers. Complete blood count (CBC), serum iron profile, serum folate levels, serum vitamin B₁₂, and CD4 count were assessed in all the samples. The CBC was performed using fully automated 3-part differential hematology analyzer, Advia centaur CP/XP (seimens) for estimation of serum vitamin B₁₂ and folate, Dade dimension/RXL (seimens) for estimation of

Serum iron and TIBC, Immulite 2000/2000 XPI (seimens) for estimation of serum ferritin, and the CD4 count was measured by flow cytometry using single-platform BD FACSCOUNT™ machine, according to the NACO guidelines.

Anaemia was defined as haemoglobin <13 g/dl (Men) and <12 g/dl (women). Leucopenia was defined as total WBC count less than 4000 cells/μl. Neutropenia was defined as absolute neutrophil count <2000 cells/μl. Lymphopenia was considered when absolute lymphocyte count <1000 cells/μl. Thrombocytopenia was defined as total platelet count < 150 × 10³/μl.^{5,6}

The results of all the patients were pooled and various haematological parameters were obtained. Data was recorded for all patients in a prescribed proforma. Among the various haematological parameters, further meticulous observation was carried out for thirteen parameters.

Statistical analysis

All the records were rechecked for their completeness. Non numeric entries were coded numerically into nominal/ordinal distribution using SPSS software version 22.0 before analysis. Categorical variables were summarized in frequency, proportions and percent distribution and Chi-square test was performed. Continuous variables were analyzed using descriptive statistics (minimum, maximum, Mean±standard deviation, or median). Mean difference between the two independent groups was analyzed by using Independent t-test after normalizing the distribution. Matched and ROC analysis were also performed to compare the accuracy level. For testing the null hypothesis, critical value for alpha was set at 0.05 (type I error) and 95% confidence limit was applied. The p value less than 0.05 was considered statistically significant.

RESULTS

Out of 109 HIV patients, 59 were male and 50 were female. Anaemia was present in 64 (58.7%) of cases. 57.6% (n=34) of male patients were anaemic and 60% (n=30) of female patients were anaemic. Among anaemic patients (n=64), normocytic normochromic anaemia was the most common type of anaemia (65.6%), followed by microcytic hypochromic anaemia (23.5%), macrocytic anaemia (7.8%) and dimorphic anaemia (3.1%) as shown in Table 1. Haematological parameters in 109 HIV patients with different CD4 groups (low CD4 and high CD4 group) were studied and compared. In patients with CD4 count less than 200 cells/mm³ (n=52), 88.5% had anaemia, 34.6% had leucopenia, 36.5% had neutropenia, 51.9% had lymphopenia and 25% had thrombocytopenia. In patients with CD4 count more than 200 cells/mm³ (n=57), 31.6% had anaemia, 21.1% had leucopenia, 24.6% had neutropenia, 24.6% had lymphopenia and 10.5% had thrombocytopenia. Observations made in HIV

patients when these two CD4 groups are compared as shown in Table 2.

The haemoglobin level and red cell indices (MCV, MCH, and MCHC) showed statistically significant correlation with CD4 counts with p value <0.05. The parameters of iron profile- serum iron, TIBC were not significantly correlated but the serum ferritin level was significantly correlated to CD4 counts with p value <0.05. Serum

folate and serum vitamin B₁₂ also didn't show significant correlation to CD4 counts. Leucopenia was found in 27.5%, Lymphopenia in 37.6%, Neutropenia in 30.3% and Thrombocytopenia 17.4% cases respectively. TLC, ANC and platelet count didn't show significant correlation with CD4 count but Absolute lymphocyte count (ALC) showed significant positive correlation with CD4 count, showing the reduction of ALC with disease progression (Table 3).

Table 1: Morphological pattern of blood picture.

Pattern of blood picture	Number of patients	Percentage (n=109)	Percentage of anaemia (n=64)
Normocytic normochromic blood picture (Normal)	45	41.3%	-
Normocytic normochromic anaemia	42	38.5%	65.6%
Microcytic hypochromic anaemia	15	13.8%	23.5%
Macrocytic anaemia	5	4.6%	7.8%
Dimorphic anaemia	2	1.8%	3.1%
Total	109	100%	100%

Table 2: Relationship between haematological parameters and CD4 groups.

Parameters	CD ₄ <200/μL (Mean±SD) n=52	CD ₄ >200/μL (Mean±SD) n=57	Chi-square	p-value
Anaemia (no. of cases)	46 (88.5%)	18 (31.6%)	36.30	<0.0001
Leucopenia (no. of cases)	18(34.6%)	12 (21.1%)	2.625	0.269
Neutropenia (no. of cases)	19 (36.5%)	14 (24.6%)	3.15	0.207
Lymphopenia (no. of cases)	27 (51.9%)	14 (24.6%)	8.68	0.013
Thrombocytopenia (no. of cases)	13 (25%)	6 (10.5%)	4.012	0.129

Table 3: Relationship between haematological parameters and CD4 counts.

Haematological variables	CD ₄ <200/μL (Mean±SD)	CD ₄ >200/μL (Mean±SD)	t-test	P-value
Hb (gm/dL)	9.60±2.55	12.94±1.58	8.29	<0.0001
MCV (fl)	86.05±11.82	91.89±6.68	3.21	0.002
MCH (pg/cell)	27.32±4.82	29.57±2.54	3.10	0.0025
MCHC%	30.92±3.25	29.56±2.56	2.46	0.016
TLC (cells/μL)	5186.25±2423.83	6014.05±2052.67	1.93	0.06
ANC (cells/μL)	2969.17±1786.27	3285.33±1367.79	1.04	0.30
ALC (cells/μL)	1422.67±865.76	1975.32±850.44	3.36	0.001
Platelets (lacs/mm ³)	2.41±1.21	2.73±1.19	1.41	0.162
S. iron (μg/dL)	71.21±34.19	83.98±41.26	1.75	0.08
TIBC (μg/dL)	249.85±115.79	284.77±86.61	1.79	0.076
S. ferritin (ng/dL)	456.89±468.53	261.07±244.39	2.77	0.007
S. folate (ng/mL)	5.10±5.15	4.51±2.83	0.75	0.455
S. vitamin B ₁₂ (pg/mL)	661.03±522.04	552.45±362.68	1.27	0.207

DISCUSSION

Haematological abnormalities are frequently encountered in HIV patients are anaemia, granulocyte disorders, thrombocytopenia, lymphomas, coagulopathies and

vascular malignancies like kaposi sarcoma. Although in the majority of cases, haematological abnormalities are detected in middle or advanced stages of HIV infection, some of these like anaemia and thrombocytopenia have been reported to occur in early stages of HIV infection⁷

The present study aims at recognizing the haematological manifestations of HIV infection which is very important with the continuing rise in the prevalence of HIV infection in a developing country like India.

Total 109 HIV-infected patients were enrolled for the study. The patients were divided into two groups according to their CD4 count: low CD4 (<200/ μ l) and high CD4 (200/ μ l). Out of 109 patients, male patients were 59 (54.1%) and female 50 (45.9%). The study showed male predominance, with male to female ratio of 1.18: 1. In the present study, we have taken 109 cases which was a low sample size. Hence, the study results may vary for sex distribution (in percentage) from the other studies.

The age of the patients ranged from 18 years to 74 years. The mean age was found to be 34.1 \pm 9.5 years. The majority of 59 (54.1%) cases were in the age group of 30 - 44 years followed by 34 (31.3%) cases in 15 to 29 years age group. This shows that the majority of patients were in reproductive age group (i.e. \leq 45 years of age). A study from India conducted by Kumawat et al, also reported 82.67% patients in the age group of 15-49 years which correlates with our study.⁸ This observation is suggesting that HIV is more common in sexually active population as sexual transmission of the disease is the commonest mode of transmission.

In this study, haematological parameters that have been included were haemoglobin (Hb) level, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), total leucocyte count (TLC), absolute neutrophil count (ANC), absolute lymphocyte count (ALC), platelet count, serum iron, total iron binding capacity (TIBC), serum ferritin, serum folate, serum vit B12 and CD4 count.

Among the total 109 cases, 64 (58.7%) patients were anaemic. Out of which (n=64), 34 male patients (57.6%) had anaemia (hb <13 g/dl) and 30 female patients (60%) had anaemia (hb <12 g/dl). The mean haemoglobin in males was 11.93 \pm 2.84 g/dl and in females was 10.67 \pm 2.32 g/dl. The finding is consistent with the observations of Mocroft et al, and Sitalakshmi et al, in which they found 59.6% and 64.2% anaemic patients respectively among their cases.^{9,10}

Among 64 anaemic patients, 42 patients (65.6%) had normocytic normochromic anaemia, 15 patients (23.5%) had microcytic hypochromic anaemia, 5 patients (7.8%) had macrocytic anaemia and only 2 patients (3.1%) had dimorphic anaemia. Rest 45 patients (41.3%) had a normocytic normochromic blood picture (Table 1). The overall picture of anaemia correlates with the studies done by Sitalakshmi et al, and Tripathi et al.^{10,11} The cumulative incidence of anaemia was higher among patients who had CD4 lymphocyte count <200 cells/ μ L as compared to patients with CD4 lymphocyte count >200 cells/ μ L, showing an inverse correlation between

anaemia and CD4 cell count ($p < 0.001$). This shows that HIV infected individuals with anaemia are at increased risk for progression to AIDS.

Mean corpuscular volume (MCV) ranged from 62.3 to 118.3 fl, mean corpuscular haemoglobin (MCH) from 16.6 to 36.3 picograms and mean corpuscular haemoglobin concentration (MCHC) from 24.8 to 35.9 g/dl. MCV in the range of 80-99 fl was seen in 87 (79.8%) cases and MCH between 27-32 pg in 74 (67.9%) cases indicating normocytic and normochromic nature of RBCs in the majority of patients.

Total leucocyte count (TLC) in this study ranged from 1.28 X 10⁹/L to 15.6 X 10⁹/L. 30 (27.5%) cases had leucopenia (<4.0 X 10⁹/L), 3 (2.8%) had leucocytosis (>10.0 X 10⁹/L) and 76 (69.7%) cases had leucocyte count within normal range. Kathuria et al, conducted a study in India on 100 HIV positive patients and found leucopenia in 25% cases.¹²

Absolute neutrophil count (ANC) ranged from 0.71 X 10⁹/L to 10.9 X 10⁹/L. Neutropenia (<2.0 X 10⁹/L) was found in 33(30.3%), 1 (0.9%) case had neutrophilia (>7.0 X 10⁹/L) and 75 (68.8%) cases had absolute neutrophil count in between 2.0 X 10⁹/L and 7.0 X 10⁹/L. Erhabor et al, conducted a study on 100 HIV positive patients and found neutropenia in 27% cases.¹³

Absolute lymphocyte count (ALC) ranged from 0.1 X 10⁹/L to 3.8 X 10⁹/L. 41 (37.6%) cases had lymphopenia (<1.0 X 10⁹/L), 11 (10.1%) cases had lymphocytosis (>3.0 X 10⁹/L) and 57 (52.3%) cases had absolute lymphocyte count within the normal range (from 1.0 X 10⁹/L to 3.0 X 10⁹/L). Kumar MB et al, conducted a study in India on 100 HIV positive patients and found lymphopenia in 38% cases.¹⁴

Platelet count ranged from 30.0 X 10⁹/L to 730 X 10⁹/L. 19 (17.4%) cases had thrombocytopenia (< 150 X 10⁹/L), 77 (70.7%) cases had platelet count in between 150X 10⁹/L and 400 X 10⁹/L, 13 (11.9%) cases had thrombocytosis (> 400 X 10⁹/L). A study conducted by Costello et al, on 925 HIV patients also found thrombocytopenia in 13% cases.³

CD4 lymphocyte count was ranged in between 18 cells/ μ l and 1120 cells/ μ l in this study. Out of 109 cases, 52 (47.7%) had CD4 count less than 200 cells/ μ l, and 57(52.3%) cases had more than 500 cells/ μ l.

Haematological parameters in 109 HIV patients with different CD4 groups (low CD4 and high CD4 group) were studied and compared. In patients with CD₄ count less than 200 cells/mm³ (n=52), 88.5% had anaemia, 34.6% had leucopenia, 36.5% had neutropenia, 51.9% had lymphopenia and 25% had thrombocytopenia. In patients with CD₄ count more than 200 cells/mm³ (n=57), 31.6% had anaemia, 21.1% had leucopenia, 24.6% had neutropenia, 24.6% had lymphopenia and

10.5% had thrombocytopenia. Observations made in HIV patients with these two CD4 groups are shown in Table 2. A study conducted by Kumar M B et al, on 100 HIV-positive patients who were assigned into two groups-group A (with CD4 counts >200 cells/mm³) and group B (with CD4 counts <200 cells/mm³).¹⁴ 50 patients were included in each group. Anaemia was the most common laboratory abnormality seen in both the groups with 70% in group A and 84% in group B, leucopenia was seen in 10% of patients in group A and 60% of patients in group B and thrombocytopenia was seen in 32% cases among group A and 78% cases among group B. Present study is consistent with the observations found by Kumar M B et al.¹⁴

Serum iron studies (serum iron, TIBC, serum ferritin), serum folate and serum vitamin B12 were also done for all the patients and finally these parameters along with haemoglobin (Hb) level, red cell indices (MCV, MCH and MCHC), TLC, ANC, ALC and platelet count were compared with different CD4 counts as shown in Table 3.

The haemoglobin level and red cell indices (MCV, MCH, and MCHC) showed statistically significant correlation with CD4 counts with p value <0.05. The parameters of iron profile- serum iron, TIBC were not significantly correlated but the serum ferritin level was significantly correlated to CD4 counts with p value <0.05. This result was similar to that reported in the study by Semba et al.¹⁵ Serum folate and serum vit. B12 also didn't show significant correlation to CD4 counts. Dikshit et al, in a study of 200 cases showed the incidence of anaemia was highest among patients who had CD4 lymphocyte count <200 cells/ μ l and was lowest with CD4 lymphocyte count >500 cells/ μ L, showing an inverse correlation between anaemia and CD4 cell count which was similar to the present study.¹⁶ They also found significant correlation of Red cell indices (MCV, MCH, MCHC) and Serum ferritin with CD4 cell counts which also correlates to our study, while no correlation between CD4 cell counts with Sr. Iron, Transferrin and TIBC was found. TLC, ANC and platelet count in our study didn't show significant correlation with CD4 count but ALC showed significant positive correlation with CD4 count, showing the reduction of ALC with disease progression (Table 3). This indicates a higher occurrence of anaemia and lymphopenia with progression of disease. Though there was a difference in mean platelet count between these two groups, it was not statistically significant, indicating occurrence of thrombocytopenia independent of disease progression.

CONCLUSION

Haematological manifestations are common in HIV-infected patients. Anaemia is the most common haematological manifestation and the most frequent form is normocytic normochromic anaemia followed by microcytic hypochromic anaemia. A proportion of patients also show leucopenia, neutropenia, lymphopenia

and thrombocytopenia. Incidence of anaemia correlates with disease progression. The present study also showed significant correlation of absolute lymphocyte count (ALC) with CD4 cell count. Thus, anaemia and absolute lymphocyte count can be used as predictors of CD4 counts and can be good clinical indicators to predict and assess the underlying immune status and the stage of the disease in centres where CD4 count evaluation is not available and may serve as useful tools in monitoring and management of HIV patients in resource poor settings, so that timely interventions can be taken.

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