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Original Research Article

Hematological Indices and Their Correlation with CD4 Cell Count among HIV Patients Attending ICTC Centre at a Tertiary Care Centre of Northern India

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Abstract

Introduction: In the resource-limited settings, especially rural and underserved areas that do not have the facilities to determine CD4 T lymphocytes count, there is a need to identify alternate markers of disease progression in HIV that are easily available, reliable, and affordable. This study aimed to determine the association of various hematological indices with CD4 T lymphocytes count.

Material and Methods: This cross sectional observational study included 150 newly diagnosed HIV seropositive patients, aged >18 years attending Integrated Counseling and Testing Centre (ICTC) at our Institute. The HIV status of these patients was confirmed at ICTC as per National AIDS Control Organization (NACO) strategy III with three rapid HIV antibody test with different antigen or principle. Complete confidentiality was maintained regarding the identity of the subjects. CD4 T lymphocytes count was estimation by CyFlow Counter (Sysmex Partec GmbH, Germany).

Results: Most of the HIV positive patients were in age group, between 26-40 years (49.33%). Male: female ratio was 2.19: 1. Mean hemoglobin level was significantly lower among patients with CD4 <200 cells/mm^3 (10.58 ± 1.34 gm/dl). The mean total leucocyte count and lymphocyte count was significantly low among patients with CD4 <200 cells/mm^3 ($p<0.001$). Mean Total Red Blood Cells (TRBC) level, hematocrit and platelet count were significantly low among patients with CD4 <200 cells/mm^3. The mean of MCV was significantly lower among patients with CD4 <200 cells/mm^3 (88.33 ± 6.2 fl) as compared to those with CD4≥500 cells/mm^3 (93.47 ± 8.11fl). The mean red cell distribution width was significantly higher among patients with CD4 <200 cells/mm^3.

Conclusion: Most abnormalities in Hematological indices could be coordinated to CD4 T lymphocyte cell counts, and these indices, specially anemia, leucopenia and lymphopenia can be used as indicators of disease progression. These indices can be useful as rough predictors of CD4 T lymphocyte cell counts in resource constraint settings where CD4 T lymphocytes and viral load estimations are not available.

Keywords: AIDS, CD4 T lymphocyte, lymphopenia, leucopenia

Introduction

Human immunodeficiency virus (HIV) infection and Acquired immunodeficiency syndrome (AIDS) is a pandemic affecting nearly all the countries of the world. [1] Progression from HIV infection to AIDS can take years. [2] Many clinical and laboratory markers have been used to estimate disease progression in HIV infection. [3] Clinical assessment lacks sensitivity for monitoring disease stage and progression of HIV infection, so it is always used in conjunction with laboratory measures. [4]

CD4+T lymphocyte serves as one such lab marker for disease progression or immune status in HIV.[5,6] The CD4 T lymphocytes cell count guides key clinical decisions ranging from when to start antiretroviral therapy (ART) to whether or not to screen for or provide prophylaxis against opportunistic infections. [7,8] Bone marrow abnormalities are detected at all stages of HIV disease, increasing in frequency as the disease progresses. Several defects in bone marrow progenitor cells have been demonstrated in patients with AIDS. [9] Haematological abnormalities frequently found in HIV-infected individuals include anaemia, granulocyte disorders, thrombocytopenia, coagulopathies, lymphomas and vascular malignancies like Kaposi sarcoma. [10] These could be due to direct effects of HIV infection, secondary infections, nutritional deficiencies, neoplasms or side effects of therapy. [11] Anaemia has been widely reported to predict a poorer prognosis for HIV- infected patients, both in terms of progression to AIDS and in survival, independent of the CD4+ T Lymphocyte count. [12]

Another abnormality that occurs in HIV patients is leukopenia occurring in 5–30% of patients with early symptomatic HIV infection and up to 70% of patients with advanced stages of AIDS. [12] Thrombocytopenia is commonly observed among patients infected by HIV and could be present as first sign of disease in approximately 5–15% of patients. [12] The burden of HIV in resource-limited countries like India is wide and a large proportion of HIV patients rely on accessing health care services in rural and underserved areas that do not have the capacity or capability to determine CD4 T lymphocyte counts. [13] So there is a need to evaluate alternate markers that are easily available, quantifiable, reliable, and affordable. [14] Various surrogate markers studied include Total Lymphocyte Count, Immunoglobulin E, β-2microglobulin and Hemoglobin. [14] Our study impresses on the need for evaluating certain economical biological markers which can be easily performed and can be useful in monitoring HIV disease progression and guide HIV disease management. Thus, present study aimed to determine the association between various haematological indices and CD4 T lymphocytes count.

Material and Methods:

This cross sectional observational study was conducted in Department of Microbiology, Sawai Man Singh Medical College, Jaipur; from May 2020 to March 2021. One hundred and fifty HIV seropositive patients, aged >18 years, who visited Integrated Counseling and Testing Centre (ICTC) at our Institute were included in the study. The HIV status of these patients was confirmed at ICTC as per National AIDS Control Organization (NACO) strategy III with three rapid HIV antibody test with different antigen or principle. Patient with any pre-existing hematological abnormalities prior to diagnosis of HIV, Cancer patients and patient on Chemotherapy or Steroids were excluded from the study.

The study was started after approval from Institutional Ethics Committee. After obtaining informed consent from the patients, the socio- demographic details, clinical sign and symptoms and history of

risk behaviour was obtained using a predesigned semi structured proforma. Complete confidentiality was maintained regarding the identity of the subjects by concealing the name and addresses of the patients from the study proforma.

Sample Collection:

Blood was collected by venepuncture with standard precautions. Five ml of blood was drawn and distributed in two K2 EDTA vials for CBC and $CD4^{+}T$ lymphocyte count. The sample was tested for CD4+T lymphocytes count estimation by CyFlow Counter (Sysmex Partec GmbH, Germany) and for complete hemogram in haematology laboratory. The tests were performed as per the standard laboratory protocols.

Statistical analyses: Data collected was entered in MS Excel Spread sheet. Nominal / categorical variables were summarized as frequency and percentage and were analyzed using Chi square test. Continuous variables were summarized as mean and standard deviation which were analyzed using ANOVA test for comparison between multiple groups (>2 groups). The p value ≤ 0.05 was taken as statistically significant. All statistical analysis was done using Epi info version 7.2.1.0 statistical software.

Results:

Most of the HIV positive patients were in age group, between 26-40 years (49.33%), followed by 41 – 55 years (28.67%). Only 16.67% were below 26 years of age, while only 5.33% patients were above 55 years of age. Most HIV patients were males (68.67%), with a male: female ratio of 2.19: 1. Most (48.67%) patients were educated up to primary, while 17.33% were illiterate. 25.33% were graduates or post graduates. The common occupations of these patients was laborer (26.67%), private or government job (17.33%), business (14.67%) and farmer (12%), while 35 (23.33%) were housewives.

Table 1: Baseline characteristics of all HIV patients

		Number of HIV positive patients	Percentage of HIV Positive patients
Age group (in years)	**<26**	25	16.67
	26-40	74	49.33
	41-55	43	28.67
	56-70	8	5.33
Gender	**Female**	47	31.33
	Male	103	68.67
Education	**Postgraduate**	11	7.33
	Graduate	27	18.00
	Secondary	13	8.67
	Primary	73	48.67
	Illiterate	26	17.33
Occupation	**Business**	22	14.67
	Job (Pvt./Govt.)	26	17.33
	Agriculture	18	12.00
	Truck Driver/Helper	4	2.67
	Laborer	40	26.67
	Student	3	2.00
	Housewife	35	23.33
	Unemployed/Retired	2	1.33

(Table 1) In present study, more than half (57.33%) HIV positive patients had CD4 count < 200 cells/mm³, followed by CD4 count of 200-499 cells/mm³ (36%), while only 10 (6.67%) had CD4 ≥500 cell/ mm³ (Figure 1).

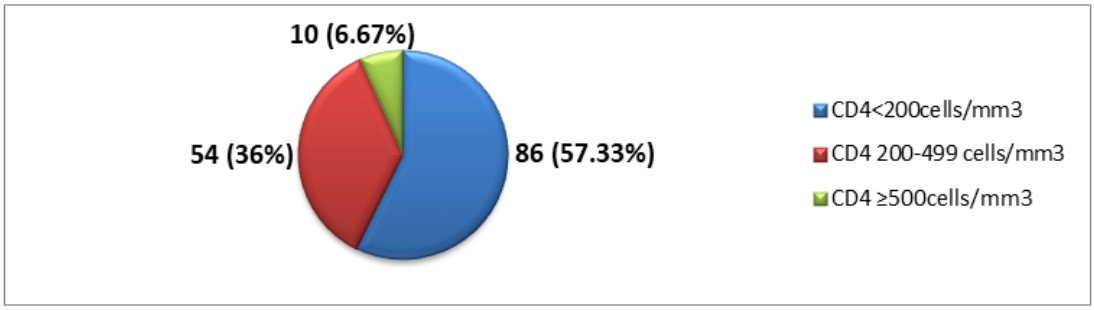

Figure 1: Distribution of study subjects according to CD4 T cells

Socio-demographic variables like age, gender, education and occupation of patients were not found to be associated with CD4 count (Table 2).

Table 2: Socio-demographic characteristics in relation to CD4 T cells count

		CD4 count (in cells/mm³)						P value
		<200		200-499		≥500		
		N	%	N	%	N	%	
Age group (in years)	**<26**	13	15.12	9	16.67	3	30	0.171
	26-40	39	45.35	32	59.26	3	30	
	41-55	31	36.05	9	16.67	3	30	
	56-70	3	3.49	4	7.41	1	10	
Gender	**Female**	27	31.40	16	29.63	4	40	0.810
	Male	59	68.60	38	70.37	6	60	
Education	**Postgraduate**	5	5.81	6	11.11	0	0	0.333
	Graduate	15	17.44	8	14.81	4	40	
	Secondary	8	9.30	5	9.26	0	0	
	Primary	39	45.35	29	53.70	5	50	
	Illiterate	19	22.09	6	11.11	1	10	
Occupation	**Business**	12	13.95	9	16.67	1	10	0.684
	Job (Pvt./Govt.)	13	15.12	11	20.37	2	20	
	Agriculture	10	11.63	7	12.96	1	10	
	Truck Driver/Helper	4	4.65	0	0.00	0	0	
	Labourer	26	30.23	11	20.37	3	30	
	Student	0	0.00	3	5.56	0	0	
	Housewife	20	23.26	12	22.22	3	30	
	Unemployed/Retired	1	1.16	1	1.85	0	0	

Mean hemoglobin level was significantly lower among patients with CD4 <200 cells/mm³ (10.58 ± 1.34 gm/dl). The mean total leucocyte count and lymphocyte count was significantly lower among patients with CD4 <200 cells/mm³ ($p<0.001$). On the contrary, neutrophil count was significantly higher among patients with CD4 <200 cells/mm³. Mean total red blood cells level and hematocrit was significantly lower among patients with CD4 <200 cells/mm³. The mean of MCV was significantly lower among patients with CD4 <200 cells/mm³ (88.33

± 6.2 fl) as compared to those with CD4 count 200-499 cells/mm^3 (91.28 ± 8.36 fl) and CD4≥500 cells/mm^3 (93.47 ± 8.11fl). The mean platelet count was significantly lower among patients with CD4 <200 cells/mm^3 (Table 3).

Table 3: Hematological characteristics in relation to CD4 T cells count

Characteristics	CD4 count (in cells/mm^3)			P value
	<200	200-499	≥500	
Hemoglobin (gm/dl)	10.58 ± 1.34	11.69 ± 1.64	11.45 ± 2.67	**<0.001**
TLC (x10³/mm³)	5.62 ± 1.69	7.52 ± 1.55	9.5 ± 1.01	**<0.001**
Neutrophil count (%)	73.76 ± 4.43	68.24 ± 2.82	66.9 ± 2.02	**<0.001**
Lymphocyte count (%)	17.34 ± 4.62	22.54 ± 2.76	24.8 ± 2.1	**<0.001**
Monocyte count (%)	7.85 ± 0.77	8.06 ± 0.88	7.2 ± 0.63	**0.009**
Eosinophil count (%)	1.06 ± 0.24	1.17 ± 0.38	1 ± 0	0.057
TRBC (million/mm³)	3.63 ± 0.58	4.14 ± 0.69	3.75 ± 0.72	**<0.001**
MCV (fL)	88.33 ± 6.2	91.28 ± 8.36	93.47 ± 8.11	**0.016**
MCH (pg)	32.32 ± 6.67	31.5 ± 4.08	32.63 ± 4.93	0.680
MCHC (gm/dl)	36.22 ± 7.18	34.22 ± 3.46	33.43 ± 2.62	0.089
RDW (%)	18.22 ± 2.82	16.42 ± 1.49	16.81 ± 3.79	**<0.001**
Hematocrit (%)	34.24 ± 4.23	39.16 ± 5.22	35.42 ± 8.59	**<0.001**
Platelet count (x10^6/ml)	2.21 ± 0.65	2.58 ± 0.52	2.34 ± 0.83	**0.004**
ESR (mm/1st hour)	37.72 ± 15.2	39.31 ± 12.22	43 ± 15.4	0.492

Discussion:

Present study attempted to make the way for better understanding of pathogenesis of HIV disease and improve laboratory testing strategies that can be used in predicting clinical stage, initiation of Highly Active Antiretroviral Therapy (HAART) and management of HIV disease.

In present study newly diagnosed HIV patients (treatment naïve) were divided into three groups based on CD4 lymphocyte count. More than half of the HIV positive patients had < 200 CD4 cells/mm³ (57.33%), followed by those with 200-499 CD4 cells/mm³ (36%). In a similar study by SS Parinitha et al, CD4 T lymphocyte count < 200 cells/mm^3 was seen in most (70%) cases followed by 200–499 cells/mm3 (21.6%) cases. [15] This distribution could be multifactorial like unawareness, lack of education, social causes, not routinely advised by clinicians and many other factors leading to relatively late presentation at health care center. In present study most HIV patients (49.33%) were aged 26-40 years. Mean age of all patients was 37.83 ± 11.54 years. Young adults have been reported to constitute majority of the HIV cases. [15]

The male: female ratio in present study was 2.19:1. In a study conducted at this center by Mo. Khalid Hassan et al, predominance of male cases can be explained due to their migration in search of work, staying away from their spouse for long periods leads to extra marital affairs resulting in acquiring HIV infection. Moreover, the male preponderance might be due to the fact that in the existing social milieu in India, females do not seek medical care due to lack of awareness and family support. [16]

In present study, mean Haemoglobin among all HIV patients was 10.97g/dl, with majority (72%) having anaemia. Past studies have reported similar results with mean haemoglobin ranging from 10.2 g/dl to 11.34 g/dl, with anaemia in up to 89% of cases. [15,17-18] Mean haemoglobin

was significantly lower among patients with CD4<200 cells/mm³, supporting findings of other studies. [15] Various factors may contribute to the development of anaemia in HIV infected patients including nutritional deficiencies, opportunistic infections, AIDS-related malignancies, drug treatment and a direct effect of HIV on the bone marrow. [12]

In present study mean total leucocyte count (TLC) was lowest in patients with <200 CD4 cells/mm³ (5.62 ± 1.69 x10³/mm³) with 16.3% having leucopenia and highest in patient with CD4 ≥500 CD4 cells/mm³ (9.5 ± 1.01 x10³/mm³). Similar to this finding a study by SS Parinitha et al also reported significantly lower mean TLC in patients with <200 CD4 cells/mm³ (5.25 ± 2.25 x10³/mm³). [15] Decrease in TLC indirectly suggestive of reduced CD4 cells in HIV patients.

In present study the mean neutrophil count was 71.31 ± 4.31%, ranging from 64 – 82% the mean lymphocyte count was 19.7 ranging from 10 – 27%. Lymphocytes count was significantly lower (p<0.001) in patients with <200 CD4 cells/mm³ (17.34 ± 4.62%) as compared to those with 200-499 CD4 cells/mm³ (22.54 ± 2.76%) and highest in patients with ≥500 CD4 cells/mm³ (24.8 ± 2.1%). SS Parinitha et al also reported similar results with lowest absolute lymphocyte count in HIV patients with CD4 < 200 cells/mm³ (1051 ±554.2) and highest in patients with CD4 ≥500 cells/mm³ (2766 ±1260.6). [15] In present study Lymphopenia (absolute lymphocyte count < 1500 cells/mm3) was found in 63.3 % HIV cases**,** Similarly studies have reported lymphopenia between 26 to 70% cases. [15,17-19] As HIV infection progresses, lymphopenia develops, resulting in a decrease in the CD4 T cell lymphocytes. [20]

In present study, the mean total red blood cells and hematocrit were found significantly lower among patients with <200 CD4 cells/mm³ as has been reported by past studies. [15,19]

Platelet count was significantly lower (p=0.004) in patients with <200 CD4 cells/mm³ (2.21 ± 0.65) and highest in patients with 200-499 CD4 cells/mm³ (2.58 ± 0.52). In a similar study by SS Parinitha et al, platelet count was lower among patients with <200 CD4 cells/mm³ (2.17 ±1.07) and highest in patients with≥500 CD4 cells/mm³ (2.8 ±1.04). [15] Disorders of hematopoiesis, opportunistic infections and increased platelet destruction caused by nonspecific immune complexes or specific anti-platelet antibodies, have been reported as possible reasons for thrombocytopenia in HIV patients. [21, 22]

Mean corpuscular volume was significantly reduced in HIV infected individuals and correlated significantly with CD4 T cells count. Red cell distribution widths (RDW) were raised significantly in patients with low CD4 T cell count, as supported by findings of other studies. [15,19] The origin of hematological disorders in HIV infection remain incompletely understood, but has been attributed to dysfunctional hematopoiesis in bone marrow caused by several factors including nutritional stress in advanced stages of HIV, suppression of marrow by invading opportunistic infections or neoplasm, chronic disease associated changes, toxic side effects of anti-retrovirals, and HIV directly also can inhibit hematopoietic precursor cells and their differentiation and development to mature blood cells. [9,10]

Conclusion:

Hematological abnormalities are very common among HIV-infected patients. Variation in most indices, were found to be associated with variations in CD4 T cell counts. Anaemia and lymphopenia can be used as predictors of CD4 T cell count in resource limited areas. These indices can be performed easily and can prove to be very economical and useful indicators of disease status in resource constraint settings where CD4 T cells and viral load

estimations are not available.

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