Hematologic Profile of HIV-Infected Antiretroviral Naive Individuals: Comparison to HIV Seronegative Individuals

[1]Srijan Srivastav, MD, DNB Pathology, Assistant Professor, Dept. of Pathology, Veer Chandra Singh Garhwali Govt. Institute of Medical Sciences and Research, Srinagar, Uttrakhand, India

[2]Kavita Mardi, MD, DNB Pathology, Professor, Dept. of Pathology, Indira Gandhi Medical college, Shimla, Himachal Pradesh, India

Corresponding Author: Srijan Srivastav, MD, DNB Pathology, Assistant Professor, Dept. of Pathology, Veer Chandra Singh Garhwali Govt. Institute of Medical Sciences and Research, Srinagar, Uttrakhand, India

Citation this Article: Srijan Srivastav, Kavita Mardi, "Hematologic Profile of HIV-Infected Antiretroviral Naive Individuals: Comparison to HIV Seronegative Individuals", IJMSIR- July - 2021, Vol – 6, Issue - 4, P. No. 53 – 60.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Hematological abnormalities are common manifestations of HIV infection. However, very few studies show comparison of hematological parameters HIV-infected antiretroviral naive and HIV seronegative individuals. This study aims to estimate the hematologic parameters in adults attending Integrated Counselling and Testing Centre (ICTC) for determining their HIV status and to compare the hematological findings in HIV-infected antiretroviral naive individuals and HIV seronegative individuals. One-year prospective study at Indira Gandhi Medical College, Shimla included 50 HIV-naive patients and 50 control patients.

The detailed history and complete clinical examination were taken. Complete hemogram, reticulocyte count, peripheral blood smear, CD4 counts, serum iron, vitamin B12 and folic acid levels were estimated in each case. Anaemia was present in 64% HIV seropositive patients.

The RBC count, total leucocyte count, absolute and differential count of polymorphs and monocytes were significantly lower in HIV positive patients when compared with seronegative group (p<0.05). Lymphocyte counts were significantly higher in HIV positive patients than in controls.

However, no statistically significant correlation was found in the mean values of haemoglobin, haematocrit, MCV, MCH, MCHC, RDW, reticulocyte count, absolute and differential eosinophil count, platelet count, serum iron, ferritin, unsaturated iron binding capacity (UIBC), folate and vitamin B12 levels in seropositive patients when compared with seronegative patients (p>0.05). The lymphocytes showed nuclear lobulation, nuclear convolution and plasmacytoid features. Neutrophils and monocytes also revealed few morphological variations in comparison to controls.

The observations from this study will guide clinicians in treatment to monitor and improve these indices.

Keywords: HIV, Hematologic, Antiretroviral, CD4.

Introduction

Hematological abnormalities are among the most common clinicopathological manifestations in HIV/AIDS. They may cause symptoms that are life-threatening and impair the quality of life of these patients. We systematically assessed 50 individuals who were diagnosed of HIV for the first time (Pre-ART or antiretroviral naive HIV), to look for various hematological manifestations and compared them with 50 HIV negative patients. The study was carried out at ICTC, IGMC, Shimla. Although many studies have been conducted on hematologic profile of HIV positive patients, only few have compared them with HIV negative patients and even fewer have included only pre-ART patients. [1-4] Furthermore, limited literature is available on the morphological changes in various cell types on peripheral blood examination in HIV positive patients.[5-9] The paucity of data from India in these respects makes this study worth for publication.

Materials and Method

A. Study Design: Prospective Case Control Study

B. Cases: The study targeted HIV positive adults which were HAART naive i.e. were first time diagnosed of HIV at ICTC, Shimla. (n=50)

C. Patient exclusion criteria: Patients less than 18 years and those on HAART were excluded from the study to rule out haematological effects of therapy.

D. Controls: Those patients who visited ICTC and were found to be HIV negative were taken as controls. (n=50). Study participants were enrolled after agreeing and signing an informed consent form.

E. Laboratory Investigations: Peripheral blood was drawn from all the study subjects under aseptic precautions into EDTA vacutainer (3ml of blood) for complete hemogram, reticulocyte count and CD4 counts and 5ml in serum vacutainer for serum iron profile, vitamin B12 and folic acid levels. A peripheral blood smear was made to study morphology of various blood cells. CD4 counts were determined using automated FACS Count Machine TM whilst haemoglobin, haematocrit, red cell indices, platelet count, total leucocyte count (TLC) and differential were determined by Haematology auto-analyser Melet Schloesing™ (MS9-3s). The normal range of various hematologic parameters were taken from Dacie and Lewis Practical Hematology.[10]Serum iron, ferritin and unsaturated iron binding capacity (UIBC) were determined quantatively in human serum and plasma (diagnostic reagent kits of Roche) on photometric systems. Serum vitamin B12 and folic acid were estimated on chemilumiscence analyser Access II of Beckman Coulter(diagnostic reagent kits of Roche).

F. Statistical analysis used: Statistical analysis was done using Epi info version 3.5.4 and SPSS version 20.0 (manufacture- IBM Corp released 2011.IBM SRSS Statistics for window version 20.0 Armonk, NY: IBM corporation).

Results

In this study cases included 30 (60%) males and 20(40%) females and control group had32(64%) males and 18(36%) females. Nineteen (38%) cases and 15 (30%) controls were below 30 years. Majority of the patients were in CDC class A1 & A2. The mean CD4 count was 306.40 ± 241.1. There was no significant difference (p value =0.41) in the mean CD4 count between males and females (Table No.1) Majority of patients (n=22; 44%) had CD 4 count < 200/ ul. Eighteen cases (36%) had CD4 count of 200-500/ul and 10 cases (20%) had CD4 count of >500/ul. The HIV positive patients had significantly lower mean RBC count, TLC, differential and absolute neutrophil and

monocyte count compared to seronegative group. However, the differential and absolute lymphocyte count was significantly higher in the cases. The

lymphocytes also showed atypical morphological features. There was no significant differences in the mean value of haemoglobin.

Table 1: CDC Class and Mean CD4 counts stratified by gender among HIV positive patients (n=50)

CDC Classification	Cases (n=50)		Male (n=28)		Female (n=22)		P value
	No.	%	No.	%	No.	%	
A1	11	22.0	7	25.0	4	18.2	0.364
A2	13	26.0	6	21.4	7	31.8	
A3	4	8.0	1	3.6	3	13.6	
B1	0	0.0	0	0.0	0	0.0	
B2	8	16.0	5	17.9	3	13.6	
B3	6	12.0	5	17.9	1	4.5	
C1	2	4.0	0	0.0	2	9.1	
C2	1	2.0	1	3.6	0	0.0	
C3	5	10.0	3	10.7	2	9.1	
Total	50	100	28	100	22	100	
Mean CD4 counts	306.40± 241.15		317.32 ± 242.61		292.50 ± 244.24		0.722

CDC-Centre for disease control and prevention haematocrit, MCV, MCH, MCHC, RDW, reticulocyte count, differential and absolute eosinophil count, platelet count, serum iron, UIBC, ferritin, folate and vitamin B12 levels in seropositive patients when compared with seronegative patients(Table No.2).Anaemia was present in 32(64%) of HIV seropositive patients and 24(48%) seronegative patients.

Most common type of anaemia observed was normocytic normochromic anaemia (43.7%) followed by microcytic hypochromic, macrocytic and lastly dimorphic anaemia seen in 28.1%, 15.6% and 12.5% of seropositive anaemic patients respectively while control group had most common microcytic hypochromic anaemia (41.7%).

Morphological abnormalities in leucocytes and platelets: Morphological abnormalities in the lymphocytes had been the most significant finding, which included nuclear lobulation, nuclear convolution and plasmacytoid and monocytoid features. (Figure1a-h) Neutrophils also showed various morphological abnormalities.

Most common were hypogranulation, hypersegmented nuclei and cytoplasmic vacuolations in descending order of frequency. (Figure2a-g) Monocytes revealed few morphological abnormalities including nuclear irregularities and cytoplasmic vacuolations. (Figure3a-d) Platelets showed giant forms. (Figure No.3h)

Discussion

A variety of hematological manifestations is seen at every stage of HIV infection and often poses a great challenge in the comprehensive management of these patients. Possible mechanisms of hematologic

abnormalities include infection of marrow mesenchymal stem cells with HIV,[5] histiocytic reaction including hemophagocytic syndrome, defects in bone marrow progenitor cells and reduced colony growth factors for multipotent hematopoietic megakaryocytic, erythroid and granulocyte-macrophage progenitor cells. [4]Among the HIV naïve and HIV negative patients 30(60%) and 32(64%) were males respectively. The results were in accordance to the studies by Dikshit et al [1] and Parintha et al. [4] The study by Amballi et al [11] found female preponderance with 59% of the patients. Majority of our patients were in CDC grade A (56%) unlike Enawgaw et al [12] and Kasthuri et al[13]in which majority were in grade B (54.5%) and Grade C (51%) respectively.

The mean CD4 count in our study was 306.40±241.15 cells /μl. It was comparable to the study by Enawgaw et al [12] who reported pre-ART counts to be 361.10± 224.40 cells /μl. The HIV positive patients had significantly lower mean RBC count when compared with seronegative group.

This was in agreement with Tagoe et al. [2] In our study, there was no significant difference in the means of hemoglobin, hematocrit, MCV, MCH, MCHC, RDW, reticulocyte count between HIV positive and HIV naive patients. Tagoe et al[2] reported significantly lower mean hemoglobin values in HIV positive patients. No

study comparing remaining parameters is available. TLC of HIV patients were found to be significantly (<0.005) lower (7.0±3.0) as compared with HIV negative controls (9.6±3.1).

This finding was similar to findings of Tagoe et al. [2] Leucocytopenia is known to increase the incidence of opportunistic infections in HIV patients. The differential neutrophil count in HIV positive patients were found to be significantly (<0.015) lower as compared to HIV negative patients. Leiderman[14] related the decrease in neutrophil count to soluble inhibitory substances produced by HIV infected cells noted to suppress neutrophils production in vitro. HIV positive patients were found to have significantly higher differential lymphocyte count (36.5±14.9), higher number of atypical lymphocytes (23.0±10.4) and a lower differential monocyte count (2.4±1.8) when compared with the HIV- naive patients.

However, in the present study, differential eosinophil count in seropositive patients was found to be insignificant when compared with the control group (p=0.515). In concordance with other studies [1, 12] no significant correlation of platelet counts with CD4 count was found in the present study (p=0.618).

Table 2: Comparison of Mean hematological parameters by HIV serostatus

Characteristic/Variable	Cases (n=50) HIV positive	Controls (n=50) HIV negative	p-value
	Mean ± S.D.	Mean ± S.D.	
Hemoglobin(g/dl)	12.6 ± 2.4	12.6±2.2	0.945
Hematocrit (%)	42.0 ± 10.1	41.8 ± 6.5	0.533
MCV(fl)	91.7 ± 7.1	90.6 ± 9.3	0.538
MCH(pg)	29.2 ± 3.6	27.8 ± 4.7	0.091

MCHC(g/l)	31.8 ± 3.5	30.6 ± 4.1	0.139
RDW (%)	12.6 ± 1.7	13.0 ± 2.2	0.336
Reticulocyte count (%)	1.3 ± 0.8	1.3 ± 0.6	0.689
RBC (million/mm^3)	4.3±0.8	4.7±0.7	0.029
TLC(/mm^3)	7.0 ± 3.0	9.6 ± 3.1	<0.001
Polymorphs (%)	56.9 ± 14.7	63.9 ± 13.6	0.015
Lymphocytes (%)	36.5 ± 14.9	29.6 ± 12.2	0.014
Monocytes (%)	2.4 ± 1.8	3.3 ± 2.4	0.024
Eosinophil (%)	3.8 ± 4.1	3.3 ± 3.6	0.515
Platelet count (/mm^3)	2.2 ± 0.8	2.4 ± 0.9	0.151
Atypical lymphocytes (%)	23.0 ± 10.4	2.9 ± 5.9	<0.001
Absolute Neutrophil Count(/mm^3)	4306.8±2742.0	6377.3±3332.9	0.001
Absolute Eosinophil Count(/mm^3)	286.2±380.2	279±346.9	0.922
Absolute Monocyte Count(/mm^3)	170.5±168.4	293.3±269.9	0.008
Serum Iron (μg/dl)	73.4 ± 35.2	74.5 ± 39.1	0.891
Serum Ferritin(ng/ml)	260.8 ± 306.4	172.7 ± 166.7	0.276
UIBC (μg/dl)	459.5 ± 515.1	291.4 ± 228.5	0.081
Serum Folate(ng/ml)	10.4 ± 5.5	8.6 ± 4.6	0.075
Serum Vit B12 (pg/ml)	326.4 ± 247.0	351.7 ± 217.0	0.587

In the present study, the mean serum iron levels were found to be (73.4 ± 35.2) μg/dl which did not correlate with CD4 counts (p=0.718). This finding was similar to study by Dikshit et al. [1] Riera et al[15] in their study on 168 HIV positive patients found elevated serum and red cell ferritin levels attributing it to worsening infections and decreasing CD4+ lymphocyte counts. In our study, elevated serum ferritin levels were found in 24%.

a. Atypical lymphocyte with cerebriform nuclei and deep basophilic cytoplasm b. Monocytoid lymphocyte c. Atypical lymphocyte with nuclear lobation and vacuolation d. Lymphocyte showing cytoplasmic projections e. Atypical lymphocyte with dumbbell shaped nucleus f. Atypical lymphocyte with nuclear lobulation g. Atypical lymphocyte with nuclear lobulation h. Lymphocyte showing intranuclear and intracytoplasmic vacuolations. (MGG; 1000 X)
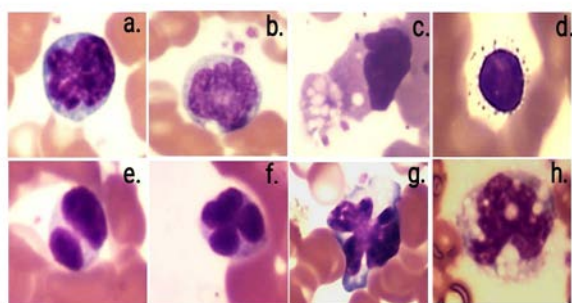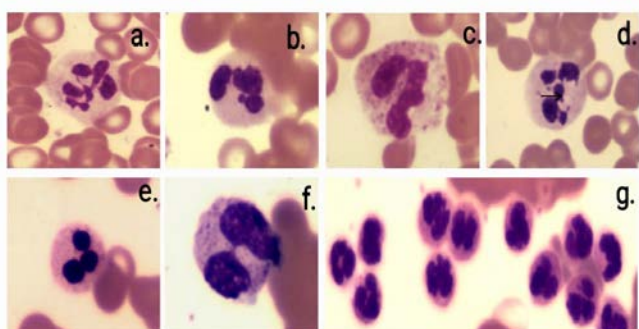

Figure 1: Morphological changes in lymphocytes

Figure 2: Morphological changes in neutrophils

a. Hypersegmented b. Hypogranular c. Toxic granules d. Pseudo-drumstick appearance (black arrow) e. Apoptotic f. Pseudo-Pelger-huet anamoly g. Agglutination (MGG; 1000 X)
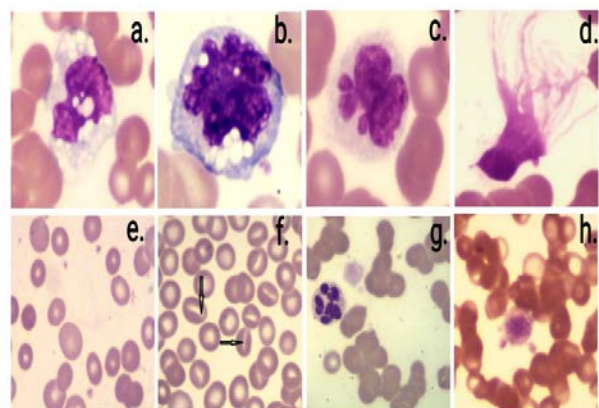

Figure 3: Morphological changes in monocytes, RBC and platelets

a. Atypical monocyte with nuclear and cytoplasmic vacuolations b. Monocyte with irregular nuclear lobation c. Atypical monocyte with nuclear protusions and vacuolation d. Smudge Cell e. Dimorphic anaemia f. Stomatocytes g. Rouleaux   h. Giant Platelets. (MGG; 1000 X)

Seropositive patients

In a study by Semeere et al [16]on ART-naive HIV positive individuals, the mean serum vitamin B12 levels were found to be 384 pg/ml. In the present study, we found a comparable value of 326.4 ± 247.0 pg/ml.

Microscopic findings

Most common type of anaemia was normocytic normochromic seen in 43.7% patients followed by microcytic hypochromic anaemia and macrocytic anaemia in 28.1% and 15.6% patients. These findings were similar to studies by consistent with Tripathi et al [6] and Kotwal et al. [5] However, in the present study, dimorphic anaemia was also found in 12.5%. Neutrophils showed various morphological abnormalities. Most common were hypogranulation, hypersegmented nuclei and cytoplasmic vacuolations in descending order of frequency. Similar abnormalities in neutrophils was noticed by Kulkarni et al. [7]

 In our study, lymphocytes were found to be atypical showing a wide range of abnormalities including bilobed, multilobulated, mushroom shaped and convoluted nuclei, cytoplasmic vacuolations and plasmacytoid features. Other investigators have also observed similar atypical lymphocytes in HIV positive patients. [8, 17]

Monocytes can also show nuclear irregularities, cytoplasmic vacuolations and atypical features in HIV patients. [18] Similar morphological abnormalities were also observed in our study.

Similar to Bamber et al [19], giant platelets were also seen in 26.0% of the patients in our study.

Conclusion

The observations from this study will serve as a guide to clinicians as treatment of these patients should include supplements to monitor and improve these indices.

References

1. Dikshit B, WanchuA, Sachdeva KR, Sharma A, Das R. Profile of hematological abnormalities of Indian HIV infected individuals. BMC Blood Disorders. 2009; 9:5-8.

2. Tagoe D, Aryee N, Evelyn A. Profiling Haematological Changes in HIV Patients Attending Fevers Clinic at the Central Regional Hospital in Cape Coast, Ghana: A Case-Control Study. Archives of Applied Science Research. 2011; 3 (5):326-31.

3. Fekene, T.E.Juhar, L.H., Mengesha C.H. et al. Prevalence of cytopenias in both HAART and HAART naïve HIV infected adult patients in Ethiopia : a cross sectional study. BMC Hematol 18,8(2018).

4. Parinitha S, Kulkarni M. Haematological changes in HIV with correlation to CD4 count. AMJ. 2012; 5 (3): 157-62.

5. Kotwal J., Singh V, Kotwal A., Dutta V, Nair V. A study of haematological and bone marrow changes in symptomatic patients with human immune deficiency virus infection with special mention of functional iron deficiency, anaemia of critically ill and hemophagocyticlymphohistiocytosis. Med J Armed Forces India. 2013; 69: 319-25.

6. Tripathi AK, Kalra P, Misra R, Kumar A, Gupta N. Study of Bone Marrow Abnormalities in Patients with HIV Disease. JAPI. 2005; 53:105-10.

7. Kulkarni C.V, Sharma Sachin, Panchonia A. Dysplastic neutrophils on peripheral blood smear hematological finding in HIV infection. Indian Journal of Basic and Applied Medical Research. 2014; 352-7.

8. Nopachai A, Christopher P, Garawacki, Stephan M. Diffuse infiltrative lymphocytosis syndrome. American Journal of Haematolgy.2004; 75:173-5.

9. Suzanne C, Hoy J, Mills J. Management of the HIV-infected Patient. In Street A, Milliken S. Hematological manifestations in HIV. Cambridge university press. 1996; 1st ed. 281-90.

10. Bain B, Bates I, Laffan A., Lewis S. Reference ranges and normal values. Dacie and Lewis Practical Hematology. Churchill Livingstone Elsevier. 2012; 11th ed. China. 14-5.

11. Amballi A, Ajibola A, Ogun S, Ogunkolo O, Salu, L. Demographic pattern and haematological profile in people living with HIV/AIDS in a university teaching hospital. Scientific Research and Essay. 2007; 2 (8): 315-8.

12. Enawgaw B, Alem M, Addis Z, Melku M. Determination of hematological and immunological parameters among HIV positive patients taking highly active antiretroviral treatment and treatment naïve in the antiretroviral therapy clinic of Gondar University Hospital, Gondar, Northwest Ethiopia: acomparative cross-sectional study.BMC Haematology. 2014; 14:8-12.

13. Kasthuri AS, Sharma S, Kar PK. A study of haematological manifestation of HIV patients. Indian J sex Trans Dis.2006;27(1):9-18.

14. Leiderman I, Greenberg M, Adelsberg B, et al. A glycoprotein inhibitor of in vitro granulopoiesis associated with AIDS. Blood. 1987; 70:1267-72.

15. Riera A, Gimferrer E, Cadafalch J, Remacha A, Martin S. Prevalence of high serum and red cell ferritin levels in HIV-infected patients. Haematologica. 1994; 79(2):165-7.

16. Semeere A, Nakanjako D, Ddungu H, Kambugu A, Manabe Y, Colebunders R. Sub-Optimal Vitamin B-12 Levels among ART-Naïve HIV-Positive Individuals in an Urban Cohort in Uganda. PLoS One. 2012; 7:7-10.

17. Betty C. Haematology in practice. In abnormalities of White blood cells: Quantitative, Qualitative and the lipid storage diseases. Davis plus. 2012; 2nd edition, 10:151-3.

Page 59

18. Treacy M, Lai L, Costello C, Clark A. Peripheral blood and bone marrow abnormalities in patients with HIV related diseases. Br J Haematol. 1987, 65(3)289-94.

19. Bamberg R, Johnson J. Segmented neutrophil size and platelet morphology in HIV/AIDS patients. Clin Lab Sci. 2002;15(1):18-22.