

Red Cells Morphological Changes on Peripheral Blood Smear Hematological Finding in HIV Infection

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Abstract: *Identify the red cells morphological changes (Peripheral blood smear) due to altered haematopoiesis resulting from HIV infection and the pathogenesis of red cells morphological changes in human immunodeficiency virus (HIV). Blood was collected in a sterile EDTA containing tube and processed following our established laboratory protocol and by universal precaution as per the guideline of National aids control organization (NACO, India). A complete blood counting including HB%, PCV, Red cell indices, platelet count and total white cell count and differential was done by automated blood cell counter analyzer of the entire patient on antiretroviral therapy. The all cell count indices including WBC count with differential and platelet count was further confirmed by manual oil immersion smear study method. Peripheral smears study was done with field A and B Stain and Leishman stain. Red blood cell changes including anisocytosis, poikilocytosis, Rouleux formation increase background staining and Occasional features of microangiopathic haemolytic anaemia (schistocyte, large helmet cell) are seen. Anisocytosis and poikilocytosis both are the most common finding in our study because HIV virus alters the homeostasis of erythropoiesis, HIV infection increase immuno-globulin protein and interfere with charge of RBC and thus increase background staining of smear.*

Keyword: Anisocytosis, Poikilocytosis, Rouleux formation, increase background staining and Occasional features of microangiopathic haemolytic anaemia (schistocyte, large helmet cell)

1. Introduction

Anaemia in patients with HIV infection who are not undergoing antiretroviral therapy with zidovudine is typically normochromic Normocytic [1] [1] [3], although a mild degree of anisocytosis and poikilocytosis is common. Macrocytosis occurs in the majority of patients treated with zidovudine. Schistocytes are prominent in the setting of thrombotic thrombocytopenic purpura, which may complicate HIV infection, In addition to being complication of the HIV infection itself.

Depressed erythropoiesis in AIDS has been suggested by a low or inappropriately normal reticulocyte count, and in HIV-infected patients the reticulocyte amount cannot be used as a reliable indicator of either hemolysis or bleeding. Similar to the anaemia of chronic disease, it is likely that inflammatory cytokines that play a role in suppressing erythropoiesis in patients of HIV infection. Tumor necrosis factor and interleukin-1 have been suppressing erythropoiesis in vitro, and both of these cytokines can be increased in HIV-infected patients. In addition, the finding on bone-marrow examination of normal to increased numbers of erythroid progenitor cells, along with a variable degree of dyserythropoiesis, has indicated that ineffective erythropoiesis may be an additional contributing factor. These all factor causes a change.

Anaemia is the most common haematological [1][7] abnormality found in children and adult with HIV infection. Indeed, anaemia was the initial manifestation of HIV infection in about 10% of children in a recent study in Italy. The importance of finding and treating anaemia [1] in adult with HIV infection is underscored by data from their study showing anaemia to be an independent prognostic factor of mortality in children with HIV infection. The

prognostic significance of anaemia at baseline is statistically significant in multiple retrospective studies in adults in the United States and Europe both in the pre-highly active antiretroviral therapy (HAART) and HAART eras.

The etiology of anaemia in adult with HIV infection is multifactorial, and managing anaemia can involve a variety of modalities. HIV infection and its direct effects on HSCs and stromal elements can lead to anaemia [5][6][7]. Opportunistic infection and myelosuppressive drugs might also cause anaemia. Another well-known cause of anaemia is pure red cell aplasia, caused by infection with parvovirus B19, and should be considered with HIV infections that have isolated anaemia. Other marrow-suppressive infections such as CMV and MAC often affect the white cell lineage, first leading to neutropenia rather than anaemia [1]. Anaemia of chronic infection as caused by these agents is Normocytic [1][3].

2. Material and Methods

Study Area and Design: The present study was conducted at the Department of Pathology MGM Medical College associated with M.Y. Hospital Indore, M.P. The study was designed as a observational hospital based study over a period of time from 2010 to 2012 years.

Ethical consideration-Detailed general, systemic examination along with complete details of patient and informed consent was obtained from all study participant do from ART Center of M.Y. Hospital Indore during the time of registration at center.

Patients selection criteria-The study targeted medically diagnosed HIV positive cases with the help of ELISA technique and confirmed by western blot under the guideline of National aids control organization (NACO, India) over period of time from 2010 to 2012. All studied 300 cases registered at ART Center and on HAART between the ages of 5 to 69 years who are schedule to visit the hospital at regular intervals of time for routine medical review was studied.

Laboratory investigations- Blood was collected in a sterile EDTA containing tube and processed following our established laboratory protocol and by universal precaution as per the guideline of National aids control organization (NACO, India). A complete blood counting including HB%, PCV, Red cell indices, platelet count and total white cell count and differential was done by automated blood cell counter analyzer of the entire patient on antiretroviral therapy. The all cell count indices including WBC count with differential and platelet count was further confirmed by manual oil immersion smear study method. Peripheral smears study was done with field A and B stain and leishman stain.

Hematological examination-

Hematological examination including HB%, PCV, Red cell indices, platelet count and total white cell count with differential count should be done on peripheral smears stained with field A and B stain and leishman stain.

3. Results

Distribution of Red Blood Cells Change Parameters

Total No. of case = 300	No.	%
Anisocytosis	128	42.66%
Poikilocytosis	259	86.33%
Rouleux formation	26	8.66%
Increase Background Staining	7	2.33%
Occasionally the blood film show features of microangiopathic Hemolytic anaemia- (Schistocyte, Large Helmet Red Cells)	5	1.66%

Distribution of Red Blood Cells Change with Parameters

Serial code	Parameters'	Male	Female
P	Normocytic	76.59%	51.78%
Q	Microcytosis	22.87%	31.25%
R	Macrocytosis	16.48%	16.96%
S	Poikilocytosis	88.29%	83.03%
T	Rouleux formation	8.51%	8.92%
U	Increase Background Staining	2.65%	1.78%
V	Occasionally the blood film show features of microangiopathic hemolytic anaemia- (Schistocyte, Large Helmet Red Cells)	0.5%	3.57%

Data analysis in following hematological parameters of red cells changes with the difference of sex distribution under

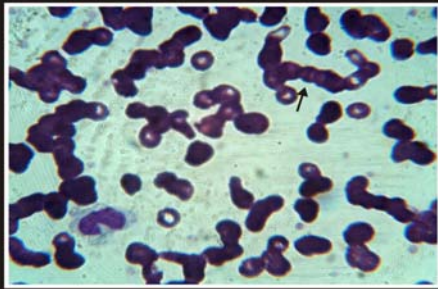
the Extended Mantel-Haenszel test for trend of chi – Squares test.

=Chi-sq.x2 value = 2.184 [DF = 1] 2-sided P = 0.139

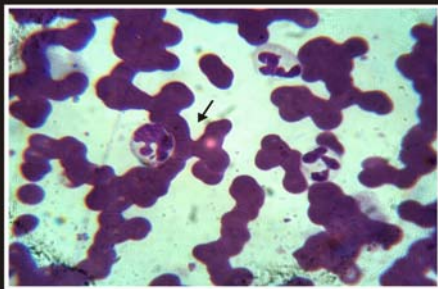
=For trend in a given direction: P = 0.070, Anisocytosis with poikilocytosis is most common morphological changes of red blood cell in HIV positive cases

4. Discussion

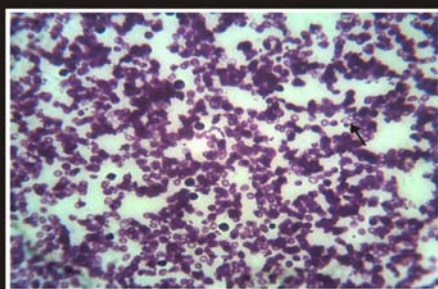
Red Blood Cells changes [4], [7]-Anisocytosis, Poikilocytosis, Rouleux formation, Increase background staining and Occasionally the blood film shows features of microangiopathic haemolytic anaemia In our study 172 cases (57.33%, n=300) shows normocytic normochromic anaemia in which male are 114 cases (76.59%, n=188) and female are 58 cases (51.78%, n=112) while 128 cases (42.66%, n=300) shows anisocytosis. There are total 78 cases (26%, n=300), in which 43 cases (22.87%, n=188) of male and 35 cases (31.35%, n=112) of female showed microcytosis. In our study 50 cases (16.66%, n=300) shows microcytosis. In which 19 cases (16.97%, n=112) of female and 31 cases (16.48%, n=188) are male. In our study 259 cases (86.33%, n=300) shows poikilocytosis in which 166 cases (88.29%, n=188) of male affected and 93 cases (83.03%, n=112) of female. In our study 26 cases (8.66%, n=300) also shows Rouleux formation in which 16 cases (8.51%, n=188) of male affected and 10 cases (8.92%, n=112) are of female. In our study 7 cases (2.33%, n=300) also shows increased background staining in which 5 cases (2.65%, n=188) are of male affected and 2 cases (1.78%, n=112) of female. In our study least common haematological finding on peripheral smear is schistocyte, only 5 cases (1.66%, n=300) show this finding. In which 1 case (0.5%, n=188) is of male and 4 cases (3.57%, n=112) of female. Similar findings were also noticed by Barbara J. Bain et al [4]. RBC changes include anisocytosis, poikilocytosis, Rouleux formation [4],[2] increase background staining and occasionally the blood film shows features of microangiopathic haemolytic anaemia (schistocyte, large helmet cell).the Upton's "N-1"chi-sq.x2 value=2.980, P=0.084 and Pearson's chi-square x2 value = 2.998 P = 0.083. Data analysis in following hematological parameters with the difference of sex distribution under the Extended Mantel-Haenszel test for trend of chi –Squares test.Chi-sq. test X2 Value = 1.388 [DF = 1] 2-sided P = 0.239For trend in a given direction: P value = 0.119.



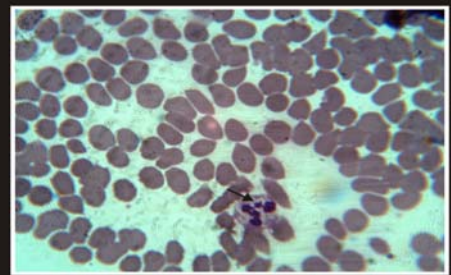
Rouleaux formation with dysplastic neutrophils & Strangely Shaped Nuclei(HP 100 X)



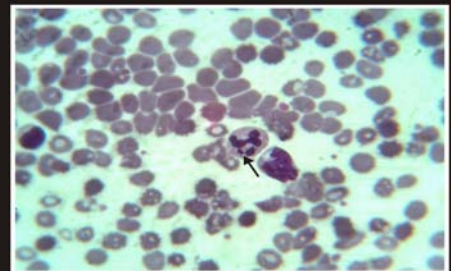
Macrocytic Anemia & Dysplastic Neutrophil with Toxic Granules (HP100X)



Microcytic hypochromic anemia (HP100X)



Detached Nuclear fragment with normocytic normochromic anemia (HP100X)



5. Conclusion

In our study of 300 cases, where 188 (62.66%, n=300) are males while 112 (37.34%, n=300) are females, highest prevalence of hematological manifestation of HIV positive patient i.e. 44% is found between 31-40 years of age. Anisocytosis with poikilocytosis is most common morphological changes of red blood cell in HIV positive cases. Out of 300 study cases normocytic normochromic anaemia is the most common type of anaemia, among both male and female. In our study, 114 cases (76.59%) of male show this type anaemia. Normocytic normochromic anaemia [1][3] along with Poikilocytosis is the most common peripheral smear change in red blood cells and presence of schistocyte is least common change in the peripheral smear. Out of 112 study cases of female normocytic normochromic anaemia is the most common type of anaemia [1], with 51.78% cases. Overall Normocytic normochromic anaemia along with Poikilocytosis is the most common peripheral smear change in red blood cells and presence of schistocyte is least common change in peripheral blood smear in HIV infection.

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Future Prospects

If any person who have these type red cells morphological changes on peripheral blood smear advised to scanning HIV infection under the NACO guide line.

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