Original Article

Hematological and Morphological changes of blood cells in HIV Patients

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ABSTRACT

Background : The Human Immunodeficiency Virus infection causes the Acquired Immunodeficiency Syndrome. Besides infectious complications, several peripheral blood cell abnormalities have been reported in HIV infection, of which anaemia and Neutropenia are reportedly the most common, which may have a considerable impact on the patient's wellbeing, and treatment.

Material and methods : Blood smears from 101 confirmed newly diagnosed HIV Positive cases were studied after taking a Written informed consent. Descriptive statistics, chi square test and contingency coefficient analysis was done using SPSS (version 16) for windows.

Results : HIV Infection affected the highly reproductive age group of 21-40 years with male predominance. Among the hematological manifestations, anemia (54.5 %) was the commonest among the patients which is predominantly Normocytic Normochromic anemia. Among the morphological changes the commonest morphological finding observed was dysplastic neutrophils.

Conclusion : There was significant statistical correlation between declining CD4 counts and Normocytic anemia, leucopenia and Thrombocytopenia. Hence all HIV Patients should be investigated for complete blood count including hematologic and morphological assessment of blood cells to reduce mortality and morbidity.

Introduction

Acquired Immunodeficiency syndrome (AIDS) – THE GRAY PLAGUE, is an acquired profound defect in T cell mediated cellular immunity that is caused by a communicable retrovirus and exposes victims to life threatening opportunistic infections and predilection to develop an infective form of Kaposi's sarcoma and certain high grade lymphomas at a relatively young age.[1] Acquired Immunodeficiency syndrome (AIDS) was first recognized in 1981 and Human immunodeficiency virus (HIV) was identified in 1983. Human Immunodeficiency Virus (HIV) infection is a global pandemic, with cases reported from virtually every country across the globe. The first case of HIV/AIDS in Bangladesh was detected in 1989. Currently, in Asia there are about 4.9 million people living with HIV, with an estimated 2.5 million in india alone.[2] HIV Infection is a multisystem disease, with hematological abnormalities amongst the most common clinicopathological manifestations with a wide range including impaired hematopoiesis, immune mediated cytopenias and coagulopathies , particularly in the later part of the disease.[3,4,5] The consequences of these hematological problems are two fold, first, they have major morbidity in themselves, adversely altering the patients quality of life. Second, they hinder the treatment of both the primary viral infection and the secondary infections and neoplastic complications.[5] The poor hematopoietic tolerance of the therapies

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often necessitates dose reductions, alteration of drug regimens, or interruption of therapies. The accurate measurements of CD4 Cell counts is essential for assessment of immune system of HIV infected person as the pathogenesis of Acquired immunodeficiency syndrome is largely attributable to the decrease in the CD4 Lymphocyte counts. In general, hematological abnormalities progress in frequency and severity with the progression of the infection from the asymptomatic HIV carrier state to the later symptomatic stages of the disease.[6] Granulocytopenias with or without lymphopenia occurs in the asymptomatic HIV carriers, children and adults with AIDS, while anemia and granulocytopenia tend to occur concomitantly with a severity that parallels the course of the HIV Infection.[7,8] Thrombocytopenia can occur independently of other cytopenias and at all stages of HIV Infection.[8] Very few studies have been done on the peripheral blood cell abnormalities of HIV Infected persons, despite them being common manifestations of HIV Infection and AIDS which may have a considerable impact on the patients well being and treatment. Hence this study was done to emphasize the need to look for hematological and morphological features in HIV Patients to improve quality of life. We also tried to evaluate the relationship between various hematological manifestations and CD4 Cell counts.

Material and methods

The prospective study comprising of 101 confirmed newly diagnosed HIV Cases was done from December 2014 to august 2016. Written informed consent is taken from all the cases. After taking a brief clinical history 1 to 1.5 ml of venous blood is collected in a sterile EDTA containing tube with universal precautions as per the guidelines of NACO, and it is processed in an Alere H automated analyser within 2 hours. The following parameters are considered for The study : Complete blood count including Hb %, PCV, Red cell indices, Platelet count, RBC Count, Reticulocyte count, WBC Count and Differential Count, CD4 Counts. Blood smears are prepared, which is routinely stained with Leishman's stain. A detailed morphological study of all the blood cell lineages were done on the peripheral smear. Smears were carefully examined for the organisms. The inclusion criteria are confirmed HIV Positive patients symptomatic and asymptomatic.The exclusion criteria are patients less than 16 Yrs, pregnant patients, patients who are on ART. We also tried to exclude the nutritional anemia in most of the cases as far as possible.

Staining Procedure

Logistics and materials :

1. Leishman stain
2. Buffered distilled water (p H 6.8-7.2)
3. Timer
4. Slide
5. EDTA blood sample

Smear preparation

1. Smear was covered with Leishman's stain
2. It was allowed to stand for 1-2 minutes
3. Without removing the stain, double the amount of buffered distilled water was added
4. Allowed it to stand for 7 minutes
5. Slide was flooded with tap water
6. Back of the slide was washed with soap and water
7. It was air dried in a tilted / upright position

A Well Stained film had the following features :

- The nuclei of leucocytes was purple
- Neutrophilic granules – tan in color
- Eosinophilic granules – red orange in color
- Basophil – dark purple granules
- Platelets – had dark lilac granules
- Cytoplasm of lymphocytes – light blue

- RBCs – pink color

Data was entered into Microsoft excel data sheet and was analyzed using SPSS 22 version software. Categorical data was represented in the form of Frequencies and proportions. Chi-square was used as test of significance. Continuous data was represented as mean and SD. ANOVA (Analysis of Variance) was the test of significance to identify the mean difference between more than two groups. Pearson Correlation was done to find the correlation between two quantitative variables. p value <0.05 was considered as statistically significant.

Results

Majority of subjects were in the age group 41 to 50 years (44.6%) and 37.6% were in the age group 31 to 40 years (Table 1). 72.3% of subjects were males and 27.7% were females.

Table 1: Age distribution of subjects

		Count	%
Age	< 30 years	10	9.9%
	31 to 40 years	38	37.6%
	41 to 50 years	45	44.6%
	> 50 years	8	7.9%
	Total	101	100.0%

Table 2: Peripheral blood smear findings in subjects

		Count	%
Anemia	Absent	46	45.5%
	Present	55	54.5%
Normocytic nemia	Absent	69	68.3%
	Present	32	31.7%
Macrocytic Anemia	Absent	82	81.2%
	Present	19	18.8%
Leucopenia	Absent	74	73.3%
	Present	27	26.7%
Leucocytosis	Absent	96	95.0%
	Present	5	5.0%
Thrombocytopenia	Absent	86	85.1%
	Present	15	14.9%

Table 3: Morphological findings in subjects

		Count	%
Macro platelets	Absent	56	55.4%
	Present	45	44.6%
Howell Jolly Bodies (RBC)	Absent	71	70.3%
	Present	30	29.7%
Atypical Lymphocytes	Absent	77	76.2%
	Present	24	23.8%
Monocytic Vacuolations	Absent	81	80.2%
	Present	20	19.8%
Detached Nuclear Fragments	Absent	50	49.5%
	Present	51	50.5%
Dysplastic Neutrophils	Absent	17	16.8%
	Present	84	83.2%
Plasmacytoid Lymphocytes		72	71.3%
		29	28.7%

Table 4: Association between CD4 count and Hematological parameters

	CD4countNew						P value
	< 200		201 to 500		> 500		
	Mean	SD	Mean	SD	Mean	SD	
RBC (x1000000/microL)	3.8	0.7	4.0	0.9	4.3	1.0	0.189
WBC (X1000/microL))	4.5	1.4	7.3	2.7	7.8	2.4	0.001*
N (%)	63.9	19.0	48.7	16.7	47.5	13.9	0.014*
L (%)	27.3	14.3	41.5	16.5	44.5	15.1	0.011*
M (%)	6.4	4.7	5.2	4.6	4.2	2.1	0.218
E (%)	1.0	0.7	3.4	3.5	2.8	2.1	0.047*
B (%)	1.2	0.6	1.1	0.5	1.0	0.4	0.391
HB (g/dl)	11.3	0.6	11.5	2.9	12.5	2.8	0.199
PLT (x 1000/microL)	149.7	70.5	244.1	108.9	247.5	47.2	0.005*

Table 5: Association between CD4 count and Peripheral blood smear in the study

		CD4count						P value
		< 200		201 to 500		> 500		
		Count	%	Count	%	Count	%	
Anemia	Absent	0	0.0%	23	44.2%	23	59.0%	0.004*
	Present	10	100.0%	29	55.8%	16	41.0%	
Normocytic Anemia	Absent	2	20.0%	37	71.2%	30	76.9%	0.002*
	Present	8	80.0%	15	28.8%	9	23.1%	
Macrocytic Anemia	Absent	10	100.0%	40	76.9%	32	82.1%	0.228
	Present	0	0.0%	12	23.1%	7	17.9%	
Leucopenia	Absent	4	40.0%	39	75.0%	31	79.5%	0.039*
	Present	6	60.0%	13	25.0%	8	20.5%	
Leucocytosis	Absent	10	100.0%	49	94.2%	37	94.9%	0.742
	Present	0	0.0%	3	5.8%	2	5.1%	
Thrombocytopenia	Absent	5	50.0%	42	80.8%	39	100.0%	<0.001*
	Present	5	50.0%	10	19.2%	0	0.0%	

There was significant association between CD4 count and anemia. i.e. with decrease in CD4 count there was increase in anemia rate.Similarly there was significant association between CD4 count and Normocytic anemia, leucopenia and Thrombocytopenia. No significant association was observed between CD4 count and Macrocytic anemia, Leucocytosis (Table 5).

Table 6: Association between CD4 count and Morphological changes in the study

		CD4countNew						P value
		< 200		201 to 500		> 500		
		Count	%	Count	%	Count	%	
Macro platelets	Absent	6	60.0%	28	53.8%	22	56.4%	0.927
	Present	4	40.0%	24	46.2%	17	43.6%	
Howell Jolly Bodies (RBC)	Absent	7	70.0%	38	73.1%	26	66.7%	0.803
	Present	3	30.0%	14	26.9%	13	33.3%	
Atypical Lymphocytes	Absent	4	40.0%	43	82.7%	30	76.9%	0.015*
	Present	6	60.0%	9	17.3%	9	23.1%	
Monocytic Vacuolations	Absent	10	100.0%	41	78.8%	30	76.9%	0.248
	Present	0	0.0%	11	21.2%	9	23.1%	
Detached Nuclear Fragments	Absent	6	60.0%	23	44.2%	21	53.8%	0.519
	Present	4	40.0%	29	55.8%	18	46.2%	
Dysplastic Neutrophils	Absent	1	10.0%	7	13.5%	9	23.1%	0.398
	Present	9	90.0%	45	86.5%	30	76.9%	
Plasmacytoid Lymphocytes	Absent	4	40.0%	39	75.0%	29	74.4%	0.07
	Present	6	60.0%	13	25.0%	10	25.6%	

There was significant association between CD4 count and atypical lymphocytes. I.e. with decrease in CD4 count there was increase in atypical lymphocytes count. No significant association was observed between other morphological changes and CD4 count (Table 6).

Table 7: Correlation between CD4 Count and Hematological parameters

	N	Pearson Correlation	P value
RBC	101	0.244**	0.014*
WBC	101	0.471**	<0.001*
Neutrophils	101	-0.368**	<0.001*
Lymphocytes	101	0.419**	<0.001*
Monocytes	101	-0.175	0.080
Eosinophils	101	-0.016	0.873
Basophils	101	0.022	0.825
Hb%	101	0.060	0.549
Platelet Count	101	0.270**	0.006*

Pearson corelation significant at p<0.05*

Significant positive correlation was observed between CD4 count and RBC, WBC, Lymphocytes and Platelet count. i.e. with decrease in CD4 count there was decrease in RBC, WBC, Lymphocytes and platelet count or vice versa.Significant negative correlation was observed between CD4 count and Neutrophils. i.e with decrease in CD4 count there was increase in Neutrophils count and vice versa (Table 7, Figure 1, Figure 2 , Figure 3, Figure 4).

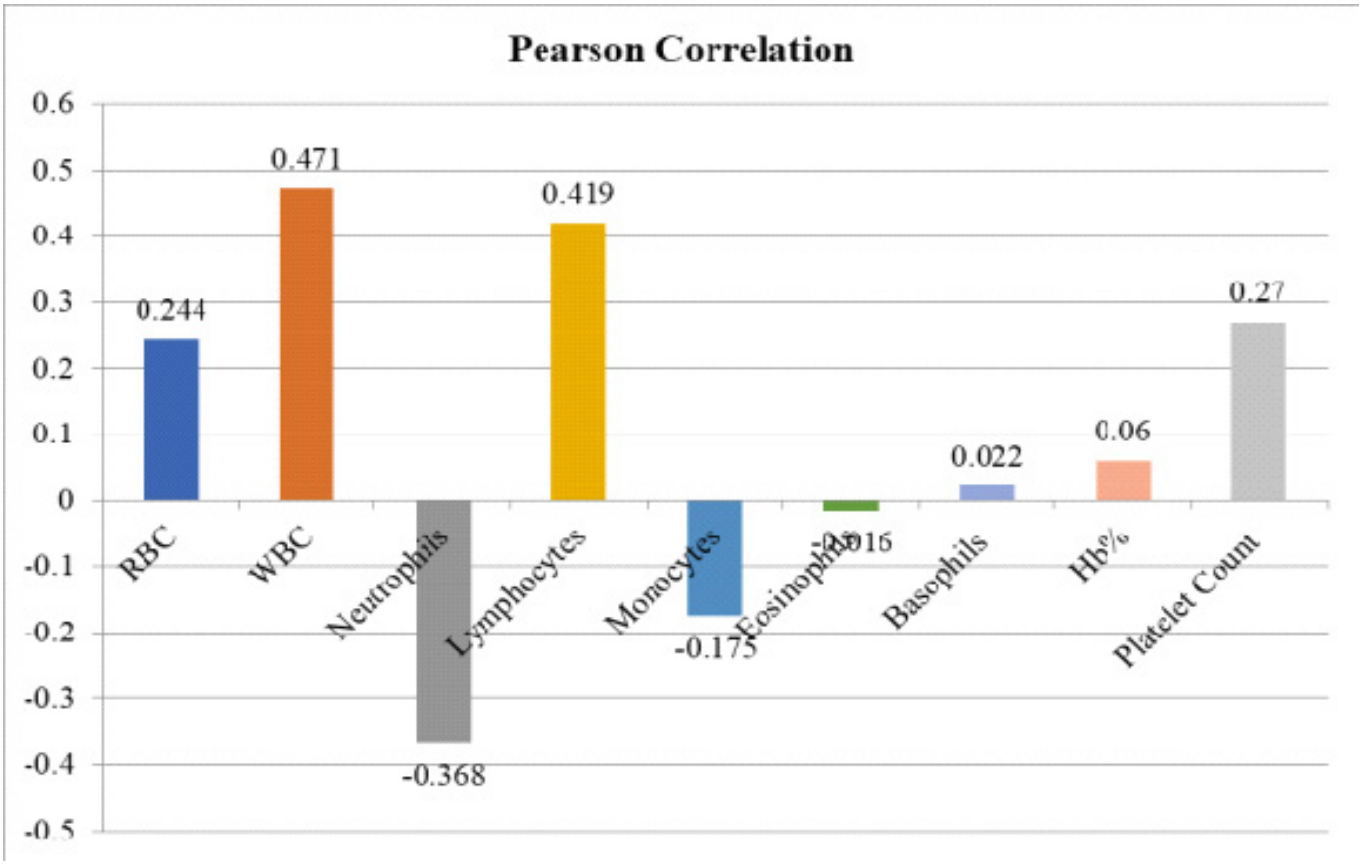

Figure 1: Bar diagram showing Correlation coefficient for hematological parameters

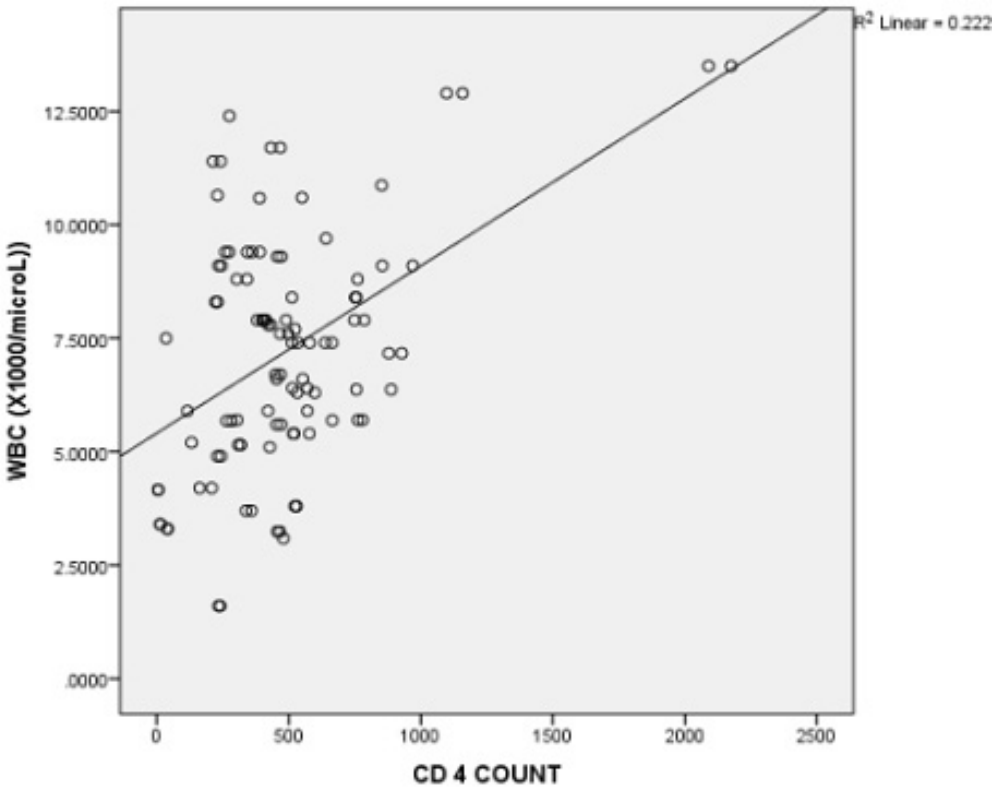

Figure 2: Scatter plot showing significant positive correlation between CD4 count and WBC

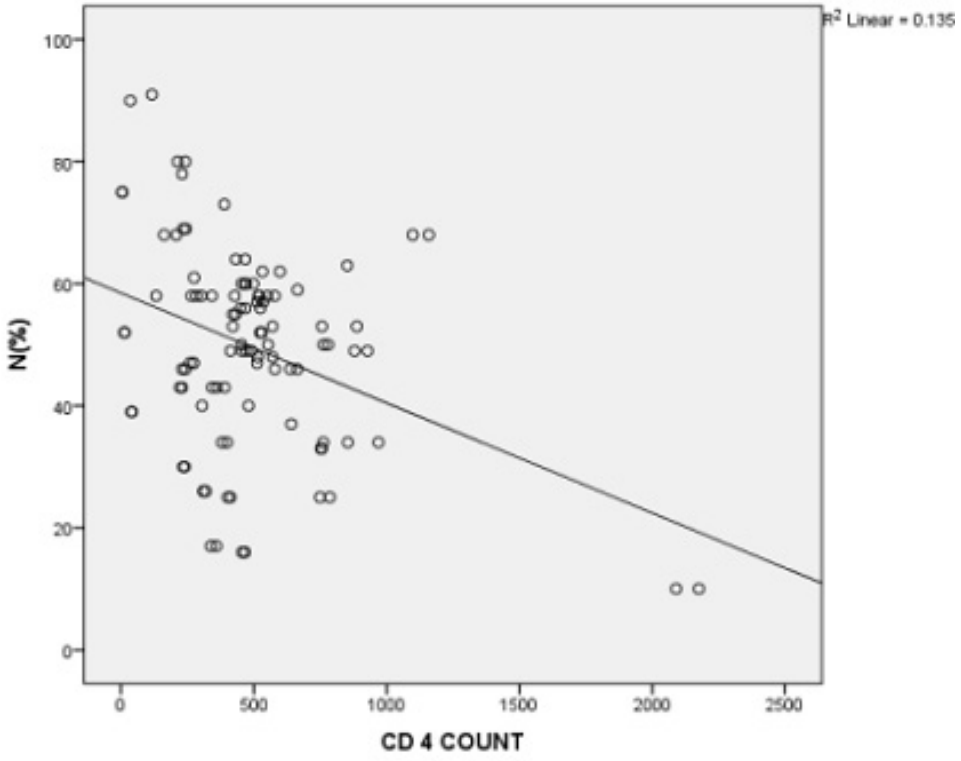

Figure 3: Scatter plot showing significant negative correlation between CD4 count and Neutrophils count

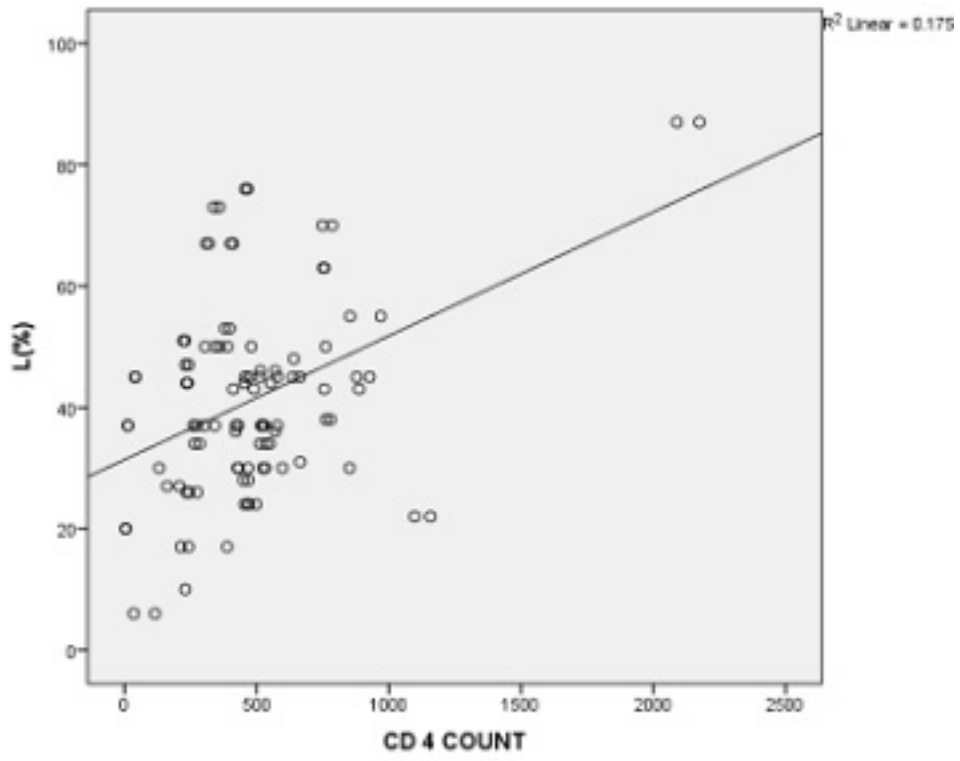

Figure 4: Scatter plot showing significant positive correlation between CD4 count and Lymphocyte count

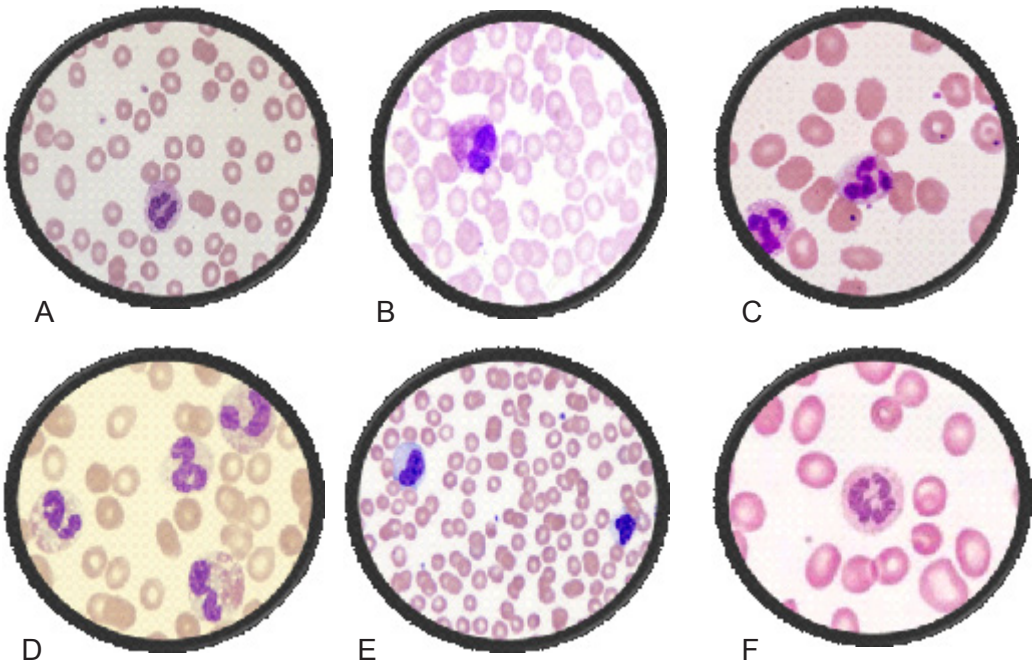

Figure 5 : Peripheral blood smear showing A) Normocytic Normochromic Anemia B) Pelger huet Anamoly C) Howell Jolly bodies D) Dysplastic Neutrophils E) Plasmacytoid lymphocytes F) Detached Nuclear fragments

Discussion

Disorders of the hematological system are common in HIV Infected patients. The hematological manifestations of HIV are varied and prevalent throughout the course of the disease. Although in the majority of the cases, hematologic abnormalities are detected in middle or advanced stages of HIV Infection, some of these like anemia and thrombocytopenia have been reported to occur in early stages of HIV Infection.[9] We evaluated 101 HIV Positive patients which were grouped into three groups according to their CD 4 Counts, those having CD 4 (<200/micro l), CD 4 (201-500 /micro l) and CD 4 (> 500/micro l). we found majority of the patients (51.5%) with CD 4 count between 201-500 /micro l, which was almost similar to the study done by Manisha et al (2002).[10]

Age Distribution

When age distribution was studied, we found 81.2% of the patients were in the sexually active age group of 21-40 years, which is almost similar to the studies done by Manisha et al (2002) [10] and Tripathi et al (2005).[11]

Sex Distribution

In present study, it was found that (n=101) male patients (73) outnumbered the female patients (28). High risk behavior and migration opportunities may be attributed to high prevalence of HIV among male patients. These results were similar to the studies done by Manisha et al (2002) 10 and Tripathi et al (2005).[11]

Hematological Manifestations

In the present study, we defined anemia according to the WHO Criteria as hemoglobin levels of < 13 gm% in males and < 12gm% in females. We found that overall prevalence of anemia was 54.5 % which was little lower as compared to studies done by Aboulafia DM et al (1991) [7], Zon Li et al (1988) [12] and Spivak JL et al (1984).[5] The lower incidence of anemia in our study can be explained on the basis of the fact that most of our study population comprised of urban population and nutritional anemia was excluded from these patients.

Total Leucocyte Count And Differential Count

In present study 26.7 % of the patients had leucocyte counts less than 4000 cells/micro l while 5 % of the patients had leucocyte counts more than 11000 cells/micro l. This is less as compared to studies done by Murphy MF et al (1987) [13], Zon Li et al (1988) [12] and Castella A et al (1985) [8] where % of leucopenic patients was 75%, 65%, and 75% patients respectively. Of the 26.7 % of the leucopenic cases in our study, 60 % of the patients CD 4 cell counts are between 201 to 500 cells/ micro l and there is a positive correlation between leucopenia and CD 4 Cell counts (p value 0.039). This implies that as the CD 4 levels increased , the total leucocyte count also followed a similar trend and showed a rise in the count.

Platelet Count

Out of 101 patients, 15 (14.9%) were having platelet counts below 1.5 lakhs/cmm and no thrombocytosis is reported in our study. When compared with the CD 4 cell count there is no single case of thrombocytopenia in patients having CD 4 Cell counts > 500 cells/micro l and 19.2 % of patients had thrombocytopenia with CD 4 Cell counts between 201-500 cells/micro l and 50% of the patients had thrombocytopenia with CD 4 Cell counts below 200 cells/micro l. This is comparitively lower than compared to other studies done by Zon Li et al (1998) [12], Murphy MF et al (1987) [13] and almost similar to the study done by Jost J et al (1988) [14]. In the present study we were able to establish a positive correlation between the platelet counts and CD 4 Cell counts (p value = < 0.001) , this implies as there is decrease is CD 4 cell count platelet count followed a similar trend and vice versa.

In our study Normocytic Normochromic Anemia accounts to majority of patients having anemia (31.7%) which is similar to the study done by Tripathi et al (2005)[11] where normocytic normochromic anemia was most common. We also found significant correlation between normocytic anemia and CD 4 Cell count (p value = 0.002).Among the morphological changes the predominant finding observed in our study was dysplastic neutrophils (83.2%) which was consistent to the findings seen in a study done by Kulkarni CV, Sachin S.[15]

Table 8 : Comparison of Morphological Patterns of blood picture in present study with other studies

Study	Parinitha et al (n=210)	Tripathi et al (n=74)	Present study
% of Normocytic Normochromic blood picture	48.1 %	17.6 %	45.5 %
% of Normocytic Normochromic Anemia	13.7 %	79.9 %	31.7 %
% of Macrocytic Anemia	7.2 %	4.1 %	18.8 %

Conclusion

In our study of 101 patients, the commonest hematological manifestation observed was anemia among which normocytic normochromic anemia predominates, followed by leucopenia and thrombocytopenia. The frequency and severity of these hematological manifestations found increased with decline in CD 4 Cell counts which can have a significant impact on clinical outcomes and quality of life. There was significant statistical correlation between between CD4 count and Normocytic anemia, Leucopenia and Thrombocytopenia. Among the morphological abnormalities, most common morphological finding observed in our study was dysplastic neutrophils followed by detached nuclear fragments with well defined cytoplasmic border, plasmacytoid lymphocytes, atypical lymphocytes. However significant statistical correlation was seen with atypical lymphocytes when compared with CD 4 Cell counts.Hence all HIV Patients should be investigated for complete blood count including hematologic and morphological assessment of blood cells and treated accordingly to reduce mortality and morbidity and to improve quality of life.

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