

## A study of hematological profile in HIV infected patients

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### Abstract

**Background:** HIV infection is associated often with a wide range of hematological abnormalities, including impaired hematopoiesis, immune mediated cytopenias and coagulopathies.

**Aims of study:** This study aims at recognizing hematological manifestation of HIV infection and to analyse hematological abnormalities in correlation with CD4 count.

**Methodology:** In this study 100 patients diagnosed as HIV seropositive by ELISA test as per WHO criteria, irrespective of their antiretroviral treatment status, attending to Department of Medicine or ART Centre, New Medical College Hospital, attached to Govt. Medical College, Kota in whom CD4 count estimation along with complete hemogram and routine investigations was done were included.

**Results:** In this study patient's age ranged from 18 to 70 years with mean age of 34 years. There was male preponderance with male to female ratio 3:2. Among the hematological abnormalities, anemia (96%) was the commonest. There was statistically significant ( $p < 0.012$ ) reduction in hemoglobin concentration with reduction in CD4 counts. The commonest type of anemias were normocytic normochromic (50%), and normocytic hypochromic (30%). Leucopenia (32%), granulocytopenia (4%), lymphopenia (38%), thrombocytopenia (40%) were the other abnormalities. But none of them showed a significant correlation with CD 4 count.

**Conclusion:** This study showed that the commonest hematological manifestation was anemia and significant number of patients showed leucopenia, lymphopenia and thrombocytopenia. The frequency and severity of these hamatological manifestations increased with decline in CD4 count and has got significant impact on clinical outcome and quality of life.

**Keywords:** HIV infected patients, hematological profile, and CD4 count

### Introduction

Acquired Immuno Deficiency Syndrome (AIDS) is caused by human immunodeficiency virus (HIV); it belongs to the family of human retroviruses (Retroviridae) and the subfamily of lentiviruses.<sup>1</sup>

HIV attacks the body's immune system. Normally, the immune system produces white blood cells and antibodies that attack viruses and bacteria. The infection fighting cells are called T-cell lymphocytes. Months to years after a person is infected with HIV, the virus destroys all the T-cell lymphocytes.<sup>2</sup>

The accurate measurement of CD4 cell counts is essential for assessment of immune system of HIV infected person as the pathogenesis of Acquired Immuno deficiency Syndrome is largely attributable to the decrease in CD4 lymphocyte counts.

HIV infection is associated often with a wide range of hematological abnormalities, including impaired hematopoiesis, immune mediated cytopenias and coagulopathies. These abnormalities may occur as a result of HIV infection itself, as sequelae of HIV-related opportunistic infections or malignancies, or as a consequence of therapies used for HIV infection and associated conditions, particularly in the later part of the disease.<sup>3,4,5</sup>

More than 50% of HIV infected patient have hematological complications. The main hematological manifestations in HIV include anemia, leucopenia, thrombocytopenia, coagulation disorders, hematological malignancies, etc.<sup>6</sup>

Anemia (Hb<10g/dl) is the most common hematological abnormality that causes symptoms in patients with HIV, affecting up to 80% of all patients. The incidence and severity increases with disease progression.<sup>7</sup> Granulocytopenia with or without lymphopenia occurs in approximately 8% of the asymptomatic HIV carriers and as many as 70% to 75% of children and adults with AIDS.<sup>8,9</sup> Thrombocytopenia (platelet count below 1.5 lakh/mm<sup>3</sup>) can occur independently of other cytopenias and at all stages of HIV infection.<sup>10</sup> Neutropenia usually occurs as HIV disease progresses and is an independent risk factor for bacterial infection in advanced HIV disease.<sup>10</sup>

According to Zon LI *et al.*, the frequency of anemia in asymptomatic HIV patients was 20% while as in with AIDS was 70% and the frequency of leucopenia was 10% among asymptomatic HIV patients while in patients with AIDS was 65%. The frequency of thrombocytopenia was 15% in

asymptomatic HIV patients while as 40% in patients with AIDS.<sup>11</sup>

According to Murphy MF *et al.*, the incidence of lymphopenia, neutropenia and thrombocytopenia in patients with AIDS was 75%, 20% and 30% respectively and in patients with asymptomatic HIV positive patients the incidence was 15%, 0% and 0% respectively.<sup>11</sup>

This study is an effort to find out various hematological abnormalities in HIV infected patients. As early identification of these hematological abnormalities would leads to appropriate planning of treatment strategies. Hence, this study was taken up in order to know the pattern of these hematologic abnormalities among people living with HIV/AIDS (PLHA).

### Materials and methods

This study was undertaken on 100 random patients and hematological parameters were analyzed in correlation with CD4 counts. All patients of >12 years of age and either sex proved to be HIV seropositive by ELISA test as per WHO criteria, irrespective of their antiretroviral treatment status, attending to Department of Medicine or ART Centre, New Medical College Hospital, attached to Govt. Medical College, Kota during the period of one year, Oct. 2012 to Sept. 2013, were included in study. All patients with HIV infection during the study period were evaluated for the conditions which could alter the hematological parameters, patients with previously known hematological disorders, congenital hematological disorders, pregnant females, intercurrent infection unrelated to HIV with significant effect on hematological profile, chronic kidney disease and if found so they were excluded from the study. All patients who included in the study were interviewed, detailed history was taken with respect to risk factors and detailed physical examination was carried out as per pre-tested proforma meeting the objective of study. Purpose of study was carefully

explained to the patients, informed consent was taken and appropriate investigation (HIV by ELISA, CD4 count by FLOW CYTOMETRY, Complete hemogram by hematology autoanalyser sysmax xs-800i, Peripheral blood film, ESR and routine) was carried out.

**Statistical Analysis**

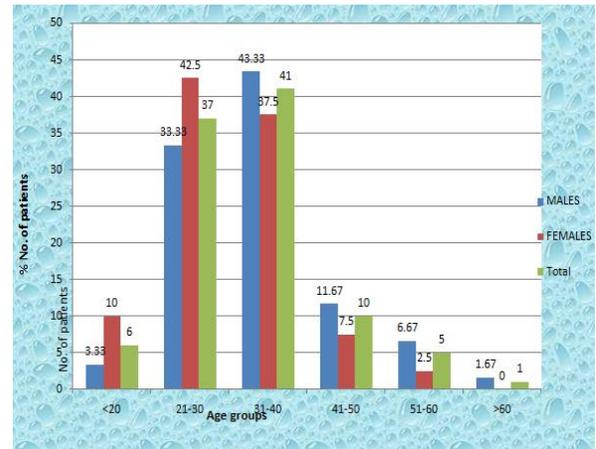
The data was collected and results were analysed by calculating percentages, the mean values, standard deviation, unpaired `t` test, Chi-square `t`test and proportion test. The proportions were compared using Chi-square test of significance. A `P` value of less than 0.05 was considered statistically significant

**Results**

Of the 100 patients studied, 60 were males against 40 females with male to female ratio 3:2. In the present study patient’s age ranged from 18 to 70 years and mean age was 34 years. Majority of cases 41(41%) were in age group of 31-40 years. As per sex males were predominantly in the age group of 31 to 40 (43.33%) whereas females were predominantly in the age group of 21 to 30 (42.5%) (Table 1).

In our study hemoglobin ranged from 3.2 to 14.5 gm% and the mean hemoglobin was 9.15 gm%. In 47 (47%) patients hemoglobin was between 9 and 13 gm%, In 41 (41%) patients hemoglobin was between 6 and 9 gm%, 8 (8%) of patients were having hemoglobin below 6 gm%. Only in 4 (4%)

of patients haemoglobin was >13 gm% (Table 2). 78 cases were having CD4 count less than 200, out of them 75 (96.15%) cases were having hemoglobin less than 13 gm% which is statistically significant (p < 0.012). The severity of anemia increased with declining CD4 count (Table 3).



**Figure: Age and sex distribution of HIV positive patients.**

In our study hematocrit ranged from 8.3 % to 45.4 % with mean hematocrit was 28.7%. 80(80%) of patients had hematocrit below 35 %. 19 (19%) of patients had hematocrit between 35-45%. Only 1 (1%) patient had hematocrit >45% (Table 2). Of the 78 cases having CD4 count < 200, 66 (84.61%) cases had hematocrit < 35% which is statistically significant (p < 0.042) (Table 3).

**Table 1: Age and sex distribution of HIV positive patients**

Age (in years)	Males (n=60)		Females (n=40)		Total (n=100)	
	Number	% of males	Number	% of female	Number	% of Total cases
≤20	2	3.33	4	10	6	6
21-30	20	33.33	17	42.5	37	37
31-40	26	43.33	15	37.5	41	41
41-50	7	11.67	3	7.5	10	10
51-60	4	6.67	1	2.5	5	5
>60	1	1.67	0	0	1	1

**Table 2: Frequency of hematological parameters percentage distribution.**

<b>Hematological Parameters</b>	<b>% of Males (n=60)</b>	<b>% of Females (n=40)</b>	<b>% of Total Cases (n=100)</b>
<b>Hemoglobin (Hb) in gm %</b>			
≤6	8.33	7.5	8
>6≤9	38.33	45	41
>9≤13	46.67	47.5	47
>13	6.67	0	4
<b>Hematocrit (HCT) in %</b>			
<35	78.33	82.5	80
35-45	20	17.5	19
>45	1.66	0	1
<b>Total Leucocyte Count (TLC) in cells/mm<sup>3</sup></b>			
<4000	35	27.5	32
4000-11000	63.63	67.5	65
>11000	1.67	5	3
<b>Neutrophil count in %</b>			
<50	3.33	5	4
50-70	65	77.5	70
>70	31.67	17.5	26
<b>Lymphocyte count in %</b>			
<20	43.33	30	38
20-40	53.33	62.5	57
>40	3.33	7.5	5
<b>Platelets count in lakhs/mm<sup>3</sup></b>			
<1.5	38.33	42.5	40
1.5-4	56.67	55	56
>4	5	2.5	4
<b>CD4 count in cells/mm<sup>3</sup></b>			
≤50	21.67	35	27
>50≤200	58.33	40	51
>200	20	25	22
<b>Type of Anemia</b>			
Normocytic normochromic	48.33	52.5	50
Normocytic hypochromic	25	37.5	30
Microcytic hypochromic	16.67	7.5	13
Macrocytic	3.33	2.5	3
Dimorphic	1.67	0	1
Pancytopenia	5	0	3

In our study total leucocyte count ranged from 420 to 14500 cells/mm<sup>3</sup>,<sup>3</sup> mean total leucocyte count was 5473 cell/mm<sup>3</sup>. 32 (32%) of cases had leucopenia (leucocyte

count below <4000 cell/mm<sup>3</sup>). Only 3 (3%) of cases had leucocytosis (Table 2). Of the 78 cases having CD4 count less than 200, out of them 26 (33.33%) were having total

leucocyte count less than 4000 which is statistically not significant ( $p < 0.122$ ) (Table 3). In our study neutrophil count ranged from 33 % to 98 % of TLC, with mean neutrophil count was 65 %. Only 4 (4%) of the cases were having granulocytopenia (neutrophil count  $<50\%$ )

(Table 2). Of 78 cases having CD4 count  $< 200$ , 2 (2.56%) cases had neutrophil count  $< 50\%$  which is statistically not significant ( $p < 0.523$ ) (Table 3).

**Table 3: hematological parameters in relation to CD4 lymphocyte count.**

Hematological Parameters	CD4 $\leq$ 200 (n=78) % of cases	CD4 $>$ 200 (n=22) % of cases	% of Total Cases (n=100)	Significance (p value)
<b>Hemoglobin (Hb) in gm %</b>				
$\leq 6$	8.98	4.54	8	p < 0.012
$>6\leq 9$	47.43	18.18	41	
$>9\leq 13$	39.74	72.72	47	
$>13$	3.84	4.54	4	
<b>Hematocrit (HCT) in %</b>				
$<35$	84.61	63.67	80	p < 0.042
35-45	15.38	31.81	19	
$>45$	0	4.54	1	
<b>Total Leucocyte Count (TLC) in cells/mm<sup>3</sup></b>				
$<4000$	33.33	27.27	32	p < 0.122
4000-11000	62.83	72.73	65	
$>11000$	3.84	0	3	
<b>Neutrophil count in %</b>				
$<50$	2.56	9.09	4	p < 0.523
50-70	67.94	77.27	70	
$>70$	29.48	13.63	26	
<b>Lymphocyte count in %</b>				
$<20$	41.02	27.27	38	p < 0.422
20-40	50.12	63.63	57	
$>40$	3.84	9.09	5	
<b>Platelets count in lakhs/mm<sup>3</sup></b>				
$<1.5$	44.87	22.72	40	p < 0.232
1.5-4	51.28	72.72	56	
$>4$	3.84	4.54	4	
<b>Type of Anemia</b>				
Normocytic normochromic	46.15	63.63	50	p < 0.023
Normocytic hypochromic	33.33	18.18	30	p < 0.032
Microcytic hypochromic	14.10	9.09	13	p > 0.05
Macrocytic	3.84	0	3	p > 0.05
Dimorphic	0	4.54	1	p < 0.05
Pancytopenia	2.56	4.54	3	p > 0.05

In our study lymphocyte count ranged from 1 % to 60 % of TLC, with mean lymphocyte count was 26 %. 38 (38%) of the cases had lymphopenia (lymphocyte count <20%) (Table 2). Of the 78 cases having CD4 count < 200, 32 cases (41.02%) had lymphocyte count < 20% which is statistically not significant ( $p < 0.422$ ) (Table 3).

In our study platelets count ranged from 0.2 to 4.62 lakhs/mm<sup>3</sup> with mean platelets counts 1.94 lakhs/mm<sup>3</sup>. 40(40%) of the cases had thrombocytopenia (platelets count <1.5 lakhs/mm<sup>3</sup>) and 4% cases had thrombocytosis (platelets count >4 lakhs/mm<sup>3</sup>) (Table 2). Of the 78 cases having CD count < 200, 35(44.87%) cases had platelets count < 1.5 lakhs/mm<sup>3</sup> which is statistically not significant ( $p < 0.232$ ) (Table 3).

In our study most common anemia was normocytic normochromic anemia (NCNCA), seen in 50% of cases, normocytic hypochromic (NCHCA) was seen in 30% of cases, microcytic hypochromic anemia (MCHCA) was seen in 13% of cases (Table 2). Of the 78 cases having CD4 count < 200, normocytic normochromic anemia (NCNCA) was present in 36 (46.15%) cases. This is statistically significant ( $p < 0.023$ ). Normocytic hypochromic anemia (NCHCA) was present in 26 (33.33%) cases which is also statistically significant ( $p < 0.032$ ) (Table 3).

In our study mean corpuscular volume (MCV) ranged from 42.2 to 132 fl with a mean of 83.6 fl, mean corpuscular hemoglobin (MCH) ranged from 12.7 to 43 pg with a mean of 27.6 pg and mean corpuscular hemoglobin concentration (MCHC) ranged from 21.3 % to 55.5 % with a mean of 32.9 %.

In our study CD4 count ranged from 5 to 772 cells/mm<sup>3</sup> and the mean CD4 count was 146 cells/mm<sup>3</sup>. 78 (78%) of cases had CD4 count less than 200 cells/mm<sup>3</sup> and 22% of cases had CD4 count more than 200 cell/mm<sup>3</sup> (Table 2).

## Discussion

In the present study age ranged from 18 to 72 years and mean age was 34 years. In the observations made by Karcher *et al.* and Khandekar *et al.* mean age was 36 years and 38 years respectively which is almost similar to the present study.<sup>12,13</sup> Majority of patients i.e., 78% were in the age group of 21-40 years in our study. Observations made by Tripathi *et al.* showed that majority of patients i.e., 63.51% were in age group of 20-40 years which is lower than our study.<sup>14</sup> In the present study there was male preponderance, with 60% males and 40 % females with male to female ratio 3:2. The observation made by Tripathi *et al.*, Khandekar *et al.* and Manisha *et al.* male to female ratio was 4:1, 2:1 and 4.8:1 respectively, which is less than the present study.<sup>13,14</sup>

In our study hemoglobin ranged from 3.2 to 14.5 gm% and the mean hemoglobin was 9.15 gm%. In observation made by Kaloutsi *et al.* hemoglobin ranged from 3.8 to 17.3 gm% with mean of 10.8 gm% which is slightly higher than observation made in the present study.<sup>15</sup>

As expected a high incidence of anemia is noted in the present study which is in accordance with other previous studies. The prevalence of anemia was astonishingly common, 96% were having hemoglobin below 13 gm%, 49% were having hemoglobin below 9 gm% and 8% were having hemoglobin below 6 gm%. This was in accordance with some studies such as Aboulafia DM *et al.*, Zon LI *et al.* Spivak JL *et al.* and Manisha *et al.* While comparing the CD4 count in relation to hemoglobin percentage, out of 96% of cases who were having hemoglobin less than 13 gm%, 75% of cases were having CD4 counts less than 200 cells/mm<sup>3</sup> and only 21% of cases were having CD4 counts more than 200 cells/mm<sup>3</sup>. 78 cases were having CD4 count less than 200, out of them 75 (96.15%) cases were having hemoglobin less than 13 gm%. There was statistically significant ( $p < 0.012$ )

reduction of hemoglobin concentration with reduction in CD4 counts. The severity of anemia increased with declining CD4 count. In our study hematocrit ranged from 8.3 % to 45.4 % with mean hematocrit 28.7 %. Observation made by Tripathi *et al.* showed a mean hematocrit of 27.36 which was slightly lower than the present study.<sup>14</sup> While comparing the CD4 count in relation to hematocrit, out of 80% of patients who were having hematocrit below 35%, 66% of cases were having CD4 counts less than 200 cells/mm<sup>3</sup> and 14% of cases were having CD4 counts more than 200 cells/mm<sup>3</sup>. 78 cases were having CD4 count less than 200, out of them 66 (84.61%) cases were having hematocrit less than 35 %. There was statistically significant ( $p < 0.042$ ) reduction of hematocrit with reduction in CD4 counts. In our study mean corpuscular volume ranged from 42.2 fl to 132 fl with a mean MCV 83.6 fl. Observation made by Tripathi *et al.* showed a mean MCV 81.81 fl which is almost similar to the present study.<sup>14</sup> Mean corpuscular hemoglobin ranged from 12.7 pg to 43 pg with a mean MCH was 27.6 pg. In observation made by Tripathi *et al.* mean MCH was 27.89 pg which is similar to present study.<sup>14</sup> In our study total leucocyte count ranged from 420 to 14500 cells/mm<sup>3</sup>, with mean total leucocyte count 5473 cells/mm<sup>3</sup>. In observation made by Kaloutsi *et al.* total leucocyte count ranged between 400 to 21000 cells/mm<sup>3</sup> with a mean total leucocyte count was 5200 cells/mm<sup>3</sup> which is almost similar to present study. Leucopenia was seen in 32% of cases. Khandekar *et al.* observed leucopenia in 17.86% of cases which is less than present study but Kaloutsi *et al.*, Zon LI *et al.*, Murphy MF *et al.* and Castella A *et al.* observed leucopenia in 50%, 65%, 75% and 75% respectively which is higher than present study.<sup>13,15,16</sup> While comparing the CD4 count in relation to total leucocyte count, out of 32% of patients who were having leucopenia, 26% of cases were having CD4 counts less than

200 cells/mm<sup>3</sup> and 6% of cases were having CD4 counts more than 200 cells/mm<sup>3</sup>. 78 cases were having CD4 count less than 200, out of them 26 (33.33%) cases were having leucopenia. There was no statistically significant ( $p < 0.122$ ) reduction in leucocytes with reduction in CD4 counts.

In our study neutrophil count ranged from 33 % to 98 % of TLC, with mean neutrophil count 65%. Only 4 (4%) of the cases were having granulocytopenia. According to Ellaurie M *et al.* and Castella A *et al.* the incidence of granulocytopenia was around 70 to 75%. While comparing the CD4 count in relation to neutrophil count, out of 4% of patients who were having granulocytopenia, 2% of cases were having CD4 counts less than 200 cells/mm<sup>3</sup> and 2% of cases were having CD4 counts more than 200 cells/mm<sup>3</sup>. 78 cases were having CD4 count less than 200, out of them 2 (2.56%) cases were having granulocytopenia. There was no statistically significant ( $p < 0.523$ ) reduction in granulocytes with reduction in CD4 counts.

In our study lymphocyte count ranged from 1% to 60% of TLC, with mean lymphocyte count 26 %. 38 (38%) of the cases were having lymphopenia as in some studies. While comparing the CD4 count in relation to lymphocyte count, out of 38% of patients who were having lymphopenia, 32% of cases were having CD4 counts less than 200 cells/mm<sup>3</sup> and 6% of cases were having CD4 counts more than 200 cells/mm<sup>3</sup>. 78 cases were having CD4 count less than 200, out of them 32 (41.02%) cases were having lymphopenia. There was no statistically significant ( $p < 0.422$ ) reduction in lymphocytes with reduction in CD4 counts.

In our study platelets count ranged from 0.2 to 4.62 lakhs/mm<sup>3</sup> with mean platelets count 1.94 lakhs/mm<sup>3</sup>. Kaloutsi *et al.* observed a range of 0.03 to 4.36 lakhs/mm<sup>3</sup> with mean of 1.96 lakhs/mm<sup>3</sup> which is almost similar to present study.<sup>15</sup> 40% of the cases were having thrombocytopenia (platelets count  $< 1.5$  lakhs/mm<sup>3</sup>). Zon LI *et*

*al.* observed 40% cases of thrombocytopenia which is similar to present study. However Karcher *et al.* reported 45% cases of thrombocytopenia which is higher than present study. Murphy MF *et al.*, Jost J *et al.*, and Kaslow RA *et al.* observed 30%, 9%, and 6.7% cases of thrombocytopenia which is lower than present study.<sup>17</sup> While comparing the CD4 count in relation to platelet count, out of 40% of patients who were having thrombocytopenia, 35% of cases were having CD4 counts less than 200 cells/mm<sup>3</sup> and 5% of cases were having CD4 counts more than 200 cells/mm<sup>3</sup>. 78 cases were having CD4 count less than 200, out of them 35(44.87%) cases were having thrombocytopenia. There was no statistically significant ( $p < 0.232$ ) reduction in platelet count with reduction in CD4 counts. Out of 4% of patients who were having thrombocytosis, 3% of cases were having CD4 counts less than 200 cells/mm<sup>3</sup> and 1% of cases were having CD4 counts more than 200 cells/mm<sup>3</sup>.

In our study most common type of anemia is normocytic normochromic anemia (NCNCA), present in 50% cases. Khandekar *et al.* observed NCNCA in 48.57% of cases, which is similar to present study. However Tripathi *et al.* observed NCNCA in 17.56% of cases, which is significantly less than present study. While comparing the CD4 count in relation to type of anemia, out of 50% of patients who were having NCNCA, 36% of cases had CD4 counts less than 200 cells/mm<sup>3</sup> and 14% of cases had CD4 counts more than 200 cells/mm<sup>3</sup>. 78 cases were having CD4 count less than 200, out of them 36(46.15%) cases were having NCNCA. There was statistically significant ( $p < 0.023$ ) prevalence of NCNCA with reduction in CD4 counts.

In our study next common type of anemia is normocytic hypochromic anemia (NCHCA), present in 30% of cases, where as Tripathi *et al.* and Khandekar *et al.* did not observe NCHCA in any patient. When compared to the CD4 count in relation to type of anemia,

out of 30% of patients who were having NCHCA, 26% of cases had CD4 counts less than 200 cells/mm<sup>3</sup> and 4% of cases had CD4 counts more than 200 cells/mm<sup>3</sup>. 78 cases were having CD4 count less than 200, out of them 26(33.33%) cases had NCHCA. There was statistically significant ( $p < 0.032$ ) prevalence of NCHCA with reduction in CD4 counts.

In our study microcytic hypochromic anemia (MCHCA) was seen in 13% of cases, where as Khandekar *et al.* and Tripathi *et al.* observed MCHCA in only 10.71% and 5.4% of cases, which is less than present study. Macrocytic anemia (MCA) was seen in 3% of cases which is almost similar to observation made by Tripathi *et al.* (4.1%), but Khandekar *et al.* observed in more cases (22.86%). 3% cases showed pancytopenia, but Spivak *et al.* reported pancytopenia in 16.6% cases which is much higher than present study.<sup>5</sup>

### Conclusion

HIV infection affects highly productive and sexually active age group (78% of cases were in age group of 21-40 years). There was male preponderance with male to female ratio 3:2.

In our study among the hematological abnormalities, anemia (96%) was the commonest. The frequency and severity of anemia worsened with declining immune status (reduction in CD4 count). There was statistically significant ( $p < 0.012$ ) reduction in hemoglobin concentration with reduction in CD4 counts. The commonest types of anemias are normocytic normochromic (50%), and normocytic hypochromic (30%). Both type of anemia were seen commonly with the worsening of immune status and there was statistically significance of both type of anemia in relation to reduction in CD4 count ( $p < 0.023$ ,  $p < 0.032$ ).

In our study leucopenia was seen in 32 % cases. There was no statistically significant ( $p < 0.122$ ) reduction in leucocytes with reduction in CD4 counts. Granulocytopenia

(neutropenia) was seen in 4% of cases. There was no statistically significant ( $p < 0.523$ ) reduction in granulocytes with reduction in CD4 counts. Lymphopenia was seen in 38% of cases. There was no statistically significant ( $p < 0.422$ ) reduction in lymphocytes with reduction in CD4 counts.

In our study thrombocytopenia was seen in 40% of cases. There was no statistically significant ( $p < 0.232$ ) reduction in platelet count with reduction in CD4 counts.

In our study 55% of cases were in CDC stage C<sub>3</sub> and 78% of cases had CD4 count <200.

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