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# A Study of Hematological Profile with Special Reference to Thrombocytopenia in HIV Infected Persons with and without Antiretroviral Therapy

Medicine

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## ABSTRACT

**Introduction:** HIV attacks the body's immune system and disables the immune system to defend the body against diseases and tumors. **Aims :** To study hematological profile of HIV infected patients and laboratory determinations of thrombocytopenia and correlate with CD4 lymphocyte count. **Methods :** This prospective non interventional observational study was carried out in KIMS, during period of 18 months from 1st October 2014 to 31st march 2016. 123 cases of HIV were included in the study. **Results :** Out of 123 patients studied,71 were males and 52 females.The mean Hb% for males in the present study is 9.7 gms% and for females is 8.1 gms%. The study have shown leucopenia in 57.7%, neutropenia in 3.3%, lymphopenia in 49.6%, and thrombocytopenia in 74% cases.Microcytic hypochromic anemia seen in 48.8% cases, dimorphic anemia in 14.6% cases and pancytopnia was seen in 13.8% cases.In males 55 cases having AIDS, 83.6% cases were having Hb% less than 13 gm%. In females 44 cases having AIDS, 90.9% cases were having Hb% less than 12 gms%. Out of 99 cases having AIDS, 57.6% cases were having leucopenia,52.3% cases had lymphopenia and 71.7% cases had thrombocytopenia. 51.5% cases had mircrocytic anemia.58.5% cases were on ART in which 75% cases had thrombocytopenia and 23.6% had leucopenia. **CONCLUSION :** Out of 123 cases, the commonest haematological manifestations found were microcytic hypochromic anemia, leucopenia and thrombocytopenia. The frequency and severity of these hematological manifestations increased with decline in CD4 count.

## KEYWORDS:

Hematological profile, HIV, thrombocytopenia, ART

### INTRODUCTION :

HIV disables the immune system to defend the body against diseases and tumors. Various infections will be able to develop, these opportunistic infections take advantage of the body's weakened immune system. These infection which normally won't cause severe or fatal health problems will eventually cause the death of the HIV patient.[1] HIV infection is a multisystem disease, hematological abnormalities are among the most common clinicopathological manifestations of HIV infection. HIV infection is associated often with a wide range of hematological abnormalities, including impaired hematopoiesis, immune mediated cytokines and coagulopathies, particularly in the later part of the disease.[2,3,4]

### AIM AND OBJECTIVES :

1. To study the hematological profile of HIV infected patients.
2. To study laboratory determinations of thrombocytopenia and correlate with CD4 lymphocyte count.
3. To study the bone marrow picture in patients of HIV with pancytopenia.

### MATERIALS AND METHODS :

**Sample size:**

One hundred and twenty three (123) cases detected to be HIV positive as per WHO criteria was taken up for study.

**Sampling method:**

Prospective non interventional, observational study.

The study was carried out as consecutive study from 1st October 2014 to 31st March 2016 by enrolling patients from Krishna Institute of medical sciences karad. Data was collected by using pre-tested proforma meeting the objectives of the study.

**Investigations:**

1. Complete haemogram including peripheral smear.
2. Reticulocyte count
3. Bone marrow aspiration/biopsy whenever indicated.
4. CD4 lymphocyte counts by FLOW CYTOMETRY by standard technique using Becton-Dickinson FAC Scan.
5. Lymphnode biopsy, ultrasound abdomen, CT scan/MRI scan if needed.

### INCLUSION CRITERIA:

HIV positive patients diagnosed as per WHO criteria irrespective of their antiretroviral treatment status admitted in Department of Medicine (MICU / Wards) in KIMS, Karad.

### EXCLUSION CRITERIA

1. Patients with previously known hematological disorders.
2. Congenital hematological disorders.
3. Age less than 18 years.
4. Patients with any other chronic illness.
5. Patients having Menorrhagia, Metrorrhagia, Polymenorrhea.

### RESULTS AND OBSERVATIONS :

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

| Age (in years) | Females (52) | | Males (71) | | Total (123) | |
|---|---|---|---|---|---|---|
| | Number | % | Number | % | Number | % |
| <20 | 3 | 5.8 | 2 | 2.8 | 5 | 4.1 |
| 21-30 | 9 | 17.3 | 5 | 7.0 | 14 | 11.4 |
| 31-40 | 17 | 32.7 | 24 | 33.8 | 41 | 33.3 |
| 41-50 | 17 | 32.7 | 27 | 38.0 | 44 | 35.8 |
| 51-60 | 4 | 7.7 | 11 | 15.5 | 15 | 12.2 |
| >60 | 2 | 3.8 | 2 | 2.8 | 4 | 3.3 |
| Total | 52 | 100.0 | 71 | 100.0 | 123 | 100.0 |

$\chi 2 = 5.27$; $p = 0.38$

**Table 2: Symptoms distribution**

| Symptoms | Number | % |
|---|---|---|
| Fatigue | 111 | 90.2 |
| Anorexia | 93 | 75.6 |
| Fever | 79 | 64.2 |
| Weight loss | 65 | 52.8 |
| Cough | 45 | 36.6 |
| Dyspnoea | 40 | 32.5 |
| Diarrhoea | 15 | 12.2 |
| Miscellaneous | 10 | 8.1 |
| Jaundice | 5 | 4.1 |

| | | |
|---|---|---|
| Bleeding | 3 | 2.4 |
| Palpitation | 3 | 2.4 |

**Table 3: Clinical Signs Distribution**

| Clinical Signs | Number | % |
|---|---|---|
| Pallor | 108 | 87.8 |
| Emaciation | 57 | 46.3 |
| Lymphadenopathy | 31 | 25.2 |
| Oral Thrush | 21 | 17.1 |
| Icterus | 17 | 13.8 |
| Edema | 13 | 10.6 |
| Clubbing | 3 | 2.4 |
| Petechiae | 1 | 0.8 |
| Bitot Spots | 1 | 0.8 |
| Cyanosis | 0 | 0 |

**Table 4: Frequency of CD4 count distribution**

| Cd4 Count (cells/mm3) | Females (52) | | Males (71) | | Total (123) | |
|---|---|---|---|---|---|---|
| | Number | % | Number | % | Number | % |
| ≤ 50 | 15 | 28.8 | 16 | 22.5 | 31 | 25.2 |
| 51-200 | 29 | 55.8 | 39 | 54.9 | 68 | 55.3 |
| > 200 | 8 | 15.4 | 16 | 22.5 | 24 | 19.5 |
| Total | 52 | 100.0 | 71 | 100.0 | 123 | 100.0 |

**$\chi 2 = 1.27; p = 0.53$**

**Table 5: Bone marrow cellularity**

| Bone Marrow | Males | Females | Total |
|---|---|---|---|
| Hypercellular | 7 | 2 | 9 |
| Hypocellular | 5 | 3 | 8 |
| Total | 12 | 5 | 17 |

**$\chi 2 = 0.02; p = 0.88$**

**Table 6: Hb% in relation to CD4 lymphocyte count in males**

| Hb (in gms %) | ≤ 50 (16) | | 51-200 (39) | | > 200 (16) | | Total (71) | |
|---|---|---|---|---|---|---|---|---|
| | Number | % | Number | % | Number | % | Number | % |
| ≤ 7 | 3 | 18.8 | 7 | 17.9 | 5 | 31.3 | 15 | 21.1 |
| 7.1-9.9 | 8 | 50.0 | 8 | 20.5 | 4 | 25.0 | 20 | 28.2 |
| 10-12.9 | 5 | 31.3 | 15 | 38.5 | 3 | 18.8 | 23 | 32.4 |
| ≥ 13 | 0 | 0.0 | 9 | 23.1 | 4 | 25.0 | 13 | 18.3 |
| Total | 16 | 100 | 39 | 100 | 16 | 100 | 71 | 100 |

$\chi 2 = 9.76; p = 0.14$

**Table 7: Hb% in relation to CD4 lymphocyte count in females**

| Hb (in gms %) | ≤ 50 (15) | | 51-200 (29) | | > 200 (8) | | Total (52) | |
|---|---|---|---|---|---|---|---|---|
| | Number | % | Number | % | Number | % | Number | % |
| ≤ 7 | 3 | 20.0 | 9 | 31.0 | 3 | 37.5 | 15 | 28.8 |
| 7.1-9.9 | 7 | 46.7 | 15 | 51.7 | 5 | 62.5 | 27 | 51.9 |
| 10-11.9 | 4 | 26.7 | 2 | 6.9 | 0 | 0.0 | 6 | 11.6 |
| ≥ 12 | 1 | 6.7 | 3 | 10.3 | 0 | 0.0 | 4 | 7.7 |
| Total | 15 | 100 | 29 | 100 | 8 | 100 | 52 | 100 |

$\chi 2 = 6.26; p = 0.39$

**Table 8: TC in relation to CD4 Count**

| Total Count (cells/mm3) | ≤ 50 (31) | | 51-200 (68) | | > 200 (24) | | Total (123) | |
|---|---|---|---|---|---|---|---|---|
| | Number | % | Number | % | Number | % | Number | % |
| < 4000 | 14 | 26.9 | 43 | 60.6 | 14 | 11.4 | 71 | 57.7 |
| 4000-11000 | 12 | 23.1 | 12 | 16.9 | 5 | 4.1 | 29 | 23.6 |
| > 11000 | 5 | 9.6 | 13 | 18.3 | 5 | 4.1 | 23 | 18.7 |
| Total | 31 | 100.0 | 68 | 100.0 | 24 | 100.0 | 123 | 100.0 |

**$\chi 2 = 5.48; p = 0.24$**

**Table 9: Neutrophil count in relation to CD4 count**

| Neutrophils (cells/mm$^3$) | ≤ 50 (31) | | 51-200 (68) | | > 200 (24) | | Total (123) | |
|---|---|---|---|---|---|---|---|---|
| | Number | % | Number | % | Number | % | Number | % |
| < 40 | 3 | 5.8 | 1 | 1.4 | 0 | 0.0 | 4 | 3.3 |
| 40-70 | 8 | 15.4 | 14 | 19.7 | 10 | 8.1 | 32 | 26.0 |
| > 70 | 20 | 38.5 | 53 | 74.6 | 14 | 11.4 | 87 | 70.7 |
| Total | 31 | 100 | 68 | 100 | 24 | 100 | 123 | 100 |

**$\chi 2 = 9.6, p = 0.04$**

**Table 10: Lymphocyte count in relation to CD4 count**

| Lymphocytes (cells/mm$^3$) | ≤ 50 (31) | | 51-200 (68) | | > 200 (24) | | Total (123) | |
|---|---|---|---|---|---|---|---|---|
| | Number | % | Number | % | Number | % | Number | % |
| <20 | 16 | 30.8 | 36 | 50.7 | 9 | 7.3 | 61 | 49.6 |
| 20-50 | 15 | 28.8 | 31 | 43.7 | 14 | 11.4 | 60 | 48.8 |
| >50 | 0 | 0.0 | 1 | 1.4 | 1 | 0.8 | 2 | 1.6 |
| Total | 31 | 100 | 68 | 100 | 24 | 100 | 123 | 100 |

**$\chi 2 = 2.95; p = 0.57$**

**Table 11: Platelet count in relation to CD4 count**

| Platelets count in (lakhs/mm$^3$) | ≤ 50 (31) | | 51-200 (68) | | > 200 (24) | | Total (123) | |
|---|---|---|---|---|---|---|---|---|
| | Number | % | Number | % | Number | % | Number | % |
| < 1.0 L | 5 | 9.6 | 11 | 15.5 | 5 | 4.1 | 21 | 17.1 |
| 1.1 L - 1.5 L | 15 | 28.8 | 40 | 56.3 | 15 | 12.2 | 70 | 56.9 |
| 1.51L - 4.0 L | 10 | 19.2 | 12 | 16.9 | 3 | 2.4 | 25 | 20.3 |
| > 4 L | 1 | 1.9 | 5 | 7.0 | 1 | 0.8 | 7 | 5.7 |
| Total | 31 | 100 | 68 | 100 | 24 | 100 | 123 | 100 |

**$\chi 2 = 5.1; p = 0.75$**

**Table 12: Type of anemia in relation to CD4 count**

| Type of anemia | ≤ 50 (31) | | 51-200 (68) | | > 200 (24) | | Total (123) | |
|---|---|---|---|---|---|---|---|---|
| | Number | % | Number | % | Number | % | Number | % |
| MHA | 13 | 41.9 | 38 | 55.9 | 9 | 37.5 | 60 | 48.8 |
| NCNC | 8 | 25.8 | 15 | 22.1 | 5 | 20.8 | 28 | 22.8 |
| DA | 3 | 9.7 | 10 | 14.7 | 5 | 20.8 | 18 | 14.6 |
| PA | 7 | 22.6 | 5 | 7.4 | 5 | 20.8 | 17 | 13.8 |
| Total | 31 | 100 | 68 | 100 | 24 | 100 | 123 | 100 |

**$\chi 2 = 7.59; p = 0.27$**

**Table 13: ART**

| ART | Females (52) | | Males (71) | | Total (123) | |
|---|---|---|---|---|---|---|
| | Number | % | Number | % | Number | % |
| No | 21 | 40.4 | 30 | 42.3 | 51 | 41.5 |
| Yes | 31 | 59.6 | 41 | 57.7 | 72 | 58.5 |
| Total | 52 | 100.0 | 71 | 100.0 | 123 | 100.0 |

$\chi 2 = 0.00; p = 0.88.$

**Table 14: Platelet count in relation to ART**

| Platelets count in lakhs/mm$^3$ | No (51) | | Yes (72) | | Total (123) | |
|---|---|---|---|---|---|---|
| | Number | % | Number | % | Number | % |
| < 1.0 L | 9 | 17.6 | 12 | 16.7 | 21 | 17.1 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 1.0 L - 1.5 L | 28 | 54.9 | 42 | 58.3 | 70 | 56.9 |
| 1.51L - 4.0 L | 10 | 19.6 | 15 | 20.8 | 25 | 20.3 |
| > 4 L | 4 | 7.8 | 3 | 4.2 | 6 | 4.9 |
| Total | 51 | 100.0 | 72 | 100.0 | 123 | 100.0 |

$\chi 2 = 1.69; p = 0.79$

**Table 15: Correlation of hematological parameters with respect to CD4 lymphocyte count**

| Hematologic parameter | CD4≤50 | CD4 51-200 | CD4 >200 | Significance |
|---|---|---|---|---|
| Hb% | | | | |
| Normal(n=15) | 1 | 12 | 4 | 0.14 |
| Decreased (n=108) | 30 | 56 | 20 | |
| TC | | | | |
| Normal(n=52) | 17 | 25 | 10 | 0.24 |
| Decreased (n=71) | 14 | 43 | 14 | |
| Neutrophil | | | | |
| Normal(n=119) | 28 | 67 | 24 | 0.06 |
| Decreased (n=4) | 3 | 1 | 0 | |
| Lymphocyte | | | | |
| Normal(n=62) | 15 | 32 | 15 | 0.41 |
| Decreased (n=61) | 16 | 36 | 9 | |
| Platelet | | | | |
| Normal(n=11) | 26 | 57 | 19 | 0.86 |
| Decreased (n=89) | 5 | 11 | 5 | |

**OBSERVATION:**

Out of 123 patients studied, 71 were males as against 52 females with a sex ratio of 1.4:1.Most of the patients were male and in the age group of 41 to 50 (38%). Predominant symptoms were fatigue (90.2%) and anorexia (75.6%) and the predominant signs were pallor (87.8%) and emaciation (46.3%). The mean Hb% for males in the present study is 9.7 gms%. In 13(18.3%) cases Hb % was more than 13gms% and 15 (21.1%) cases were having Hb% below 7 gms%. The mean Hb% for females in the present study is 8.1 gms%.In 4(8%) cases Hb% was above 12gms% and 15(28.8%) cases were having Hb% below 7 gms%. The total count ranged from 1300 cells/$mm^3$ to 22940 cells/$mm^3$, with prevalence of leucopenia in 71(57.7%) cases. The neutrophil count ranged from 38% to 89% of TLC, with only 4(3.3%) cases having neutropenia. The lymphocyte count ranged from 1 to 55% of TLC with prevalence of lymphopenia in 61 (49.6%) cases. The platelet count ranges from 0.6 to 6lakhs/$cmm^3$ with prevalence of thrombocytopenia was in 91(74%) cases. The CD4 count varied widely from 5 to 701. The most common anemia was microcytic hypochromic anemia seen in 60(48.8%) cases, normocytic normochromic anemia in 28(22.8%) cases, dimorphic anemia in 18(14.6%) cases and pancytopnia was seen in 17(13.8%) cases. Bone marrow study was done in 17 cases out of which 9 cases were having hypercellular marrow. Out of 52 females, 44 cases having CD4 count < 200, 40 (90.9%) cases were having Hb% less than 12 gms%. The mean Hb in the present study was 8.1gms%. Out of 71 males, 55 cases having CD4 count < 200, 46(83.6%) cases were having Hb% less than 13 gm%.The mean Hb in the present study was 9.7gm%.Out of 99 cases having CD4 count <200, 57(57.6%) cases were having leucopenia(< 4000), 4(4.5%) cases had neutropenia (<40) ,52(52.3%)cases had lymphopenia(<20), 71(71.7%) cases had thrombocytopenia(< 1.5 lakhs/$mm^3$).Out of 99 cases having CD4 count <200,51(51.5%) cases had mircrocytic hypochromic anemia. Out of 123 patients 72(58.5%) cases were having ART. Out of which 31(59.6%) were females and 41(57.7%) were males Of the 72 cases having ART(TLE REGIMEN), 54(75%) cases had thrombocytopenia (< 1.5 lakhs/mm3).Of the 72 cases having ART 29(23.6%) cases had leucopenia.

**DISCUSSION:**

**AGE AND SEX DISTRIBUTION**

**Table 16: Sex distribution of cases in various studies in relation to present study**

| Sex | Anil et al[5] | SS Parinitha et al[6] | Rajeev et al[7] | Present study n =123 |
|---|---|---|---|---|
| Males | 70% | 68% | 90% | 58% |
| Females | 30% | 32% | 10% | 42% |

The result of the data analysis obtained shows a predominance of males amongst 123 patients that is males constituted 57.7% (n=71).The patient age in the present study ranged from 18 to 75 years. Coming to the age distribution about 35.8% of the patients fall in the age group of 41 to 50 years. In the present study compared to male age distribution females were younger, 55% of them were below age of 40 years. There was no statistical significance regarding particular sex and age distribution (p=0.38).

**SYMPTOMS AND SIGNS**

Among the various symptoms, the most common symptom was fatigue (90%) and anorexia (76%). This prevalence of symptoms is in par with the earlier studies. Among the signs, pallor was the most common sign present in 88% of the patients, next emaciation was present in (46%). The increased prevalence of fever, fatigue and weight loss in the present study could possibly be due to severity of the illness.

**HEMATOLOGICAL MANIFESTATIONS**

As expected a high incidence of anemia is noted in the present study which is in accordance with other previous studies.The multifactorial origin of anemia in HIV disease complicates its differential diagnosis and treatment.

**ANEMIA**

**Table 17: Percentage of anemia in various studies**

| Study | Anil et al | | SS Parinitha et al | | Vanisri et al[8] | | Present study | |
|---|---|---|---|---|---|---|---|---|
| | Number | % | Number | % | Number | % | Number | % |
| % of anemia | 93 | 93 | 210 | 84 | 65 | 65 | 123 | 86 |

The prevalence of anemia was common, about 86% were having hemoglobin below 13 gm% and about 16% were having hemoglobin below 6 gm%. This was in accordance with some studies such as Anil et al, SS Parinitha et al and Vanisri et al when compared to the CD4 count in relation to hemoglobin percentage, about 80.5% of cases having Hb% less than 13 gms% were having CD4 counts ≤200 cells/$mm^3$ and only 24 cases were having CD4 counts more than 200 cells/mm3. There was no statistical significance in reduction of hemoglobin with worsening immune status (reduction in CD4 count), (p=0.09)

**TOTAL LEUCOCYTE COUNT AND DIFFERENTIAL COUNT**

**Table 18: Percentage of total leucocyte counts in various studies**

| Study | Anil et al | | SS Parinitha et al | | Rajeev et al | | Present study | |
|---|---|---|---|---|---|---|---|---|
| | Number | % | Number | % | Number | % | Number | % |
| % of Leucopenia | 19 | 19 | 52 | 20.8 | 4 | 18.2 | 71 | 58 |

In the present study about 58% of the cases were having leucopenia which is far more compared to other studies and 23 cases were having leucocytosis because of the severity of the disease in the study. 57 cases of leucopenia had CD4 counts less than 200 cells/$mm^3$ definitely showing the severity of the disease.According to Anil, SS Parinitha and Rajeev et al. the incidence of neutropenia was around 18-21%.Out of 71 patients of leucopenia 57 were having CD4 counts less than 200 cells/$mm^3$. Lymphopenia was seen in 50% (n = 61) of cases of which except 52 cases all were having CD4 counts less than 200 cells/mm3.There is no significant incidence in increase or decrease TLC in either of the sexes (p=0.44).Similarly there was no

statistical significance in neutrophil count changes with particular sex (p=0.38) and in lymphocyte count changes with particular sex (p=0.09)

**PLATELET COUNT**

**Table 19: Percentage of platelet counts in various studies**

| Study | Anil et al | | SS Parinitha et al | | Vanisri et al | | Present study | |
|---|---|---|---|---|---|---|---|---|
| | Number | % | Number | % | Number | % | Number | % |
| % of anemia | 23 | 23 | 45 | 18 | 15 | 15 | 91 | 74 |

Out of 123 patients,91(74%) were having platelet counts below 1.5 lakhs/$mm^3$. In relation to the CD4 count 71(78%) of the cases were having CD4 count below 200 cells/$mm^3$. This is in accordance with some studies. In other studies mentioned above the prevalence of thrombocytopenia is believe to be present in 15-23% of HIV infected persons. In a multicenter AIDS cohort study of 1500 HIV positive individuals, 6.7% had platelet counts less than 1.5lakhs/$mm^3$ on at least one semiannual visit. But the prevalence of thrombocytopenia in the patients with HIV/AIDS has been variably reported in 13 to 61% cases. There was no statistical significance in platelet count changes and particular sex (p=0.2).

**BONE MARROW CELLULARITY:** Bone marrow study was done in 17 cases out of which 9 cases were having hypercellular marrow and 8 were hypocellular bone marrow.

**TYPE OF ANEMIA**

**Table 20: Percentage of anemia in various studies**

| Types of Anemia | Anil et al | SS Parinitha et al | Debarshi et al[9] | Present study |
|---|---|---|---|---|
| MHA | 73(73) | 18(10.8) | 58(60.4) | 60(48.8) |
| NCNC | 17(17) | 101(60.8) | 38(39.6) | 28(22.8) |
| DA | 10(10) | 47(28.3) | 0(0) | 18(14.6) |
| PA | 0(0) | 0(0) | 0(0) | 17(13.8) |

The commonest anemia in the present study was microcytic hypochromic anemia (49%) as compared to the previous studies. Normocytic normochromic anemia was found in 23% in the present study. 15% cases of dimorphic anemia and 14% cases of pancytopenia were seen. Microcytic hypochromic anemia varied from 10% to 73 % in earlier studies.

**SUMMARY :**

Peripheral and bone marrow abnormalities are common in HIV related disease and has significant impact on clinical outcomes and quality of life (QOL).In the present study, out of 123 patients, the commonest haematological manifestations found were microcytic hypochromic anemia, leucopenia and thrombocytopenia. The variation in the prevalence of hematological abnormalities in different stages of disease are due to number of factors which includes – CD4 count, clinical disease status, drug therapy, opportunistic infections and malignancy. HIV infection affected the highly productive age group of 41-50 years of age (36%) and predominantly males (58%) in the present study. The most common symptom was fatigue (90%) and anorexia (76%), and among the signs pallor (88%) and emaciation (46%) were common. This may be due to the advanced clinical disease status and worsening immunity (88% of cases were in clinical stages III or IV). But there was no statistical significance in relation to CD4 count. Among the hematological manifestations, anemia (88%) was the commonest. The frequency and severity of anemia worsened with declining immune status (CD4 count). The commonest type of anemia in present is microcytic hypochromic (49%), which is at par with the earlier studies. Normocytic normochromic and normocytic hypochromic anemia were seen commonly with the worsening of immune status and clinical stage. But there was no statistical significance of any particular anemia in relation to reduction in CD4 count. Leucopenia was seen in 71cases (58%) But there was no statistical significance with CD4 count (p = 0.44). Thrombocytopenia was seen in 71(71.7%)cases in correlation with CD4 count < 200 cells/$mm^3$. But there was no statistical significance (p = 0.75). Bone marrow study showed 9 cases were having hypercellular marrow and 8 were having hypocellular bone marrow.

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