Original Article

A study of Haematological Profile in Human Immune Deficiency Virus Infection: Correlation with CD4 Counts

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ABSTRACT

Introduction: Hematologic defects are a common complication of human immunodeficiency virus (HIV) infection and result from several influences on the hematopoietic tissue. These abnormalities reflect the underlying immune status and may be prevented or corrected by the use of highly active antiretroviral therapy (HAART). Anemia commonly occurs during HIV infection and has been associated with increased progression to acquired immune deficiency syndrome (AIDS) and reduced survival.

Aims: The aim of this study was to study the hematologicalprofile in HIV patients, to evaluate the various hematological parameters and their association with CD4 counts.

Methods: One hundred HIV-positive patients, symptomatic as well as asymptomatic, diagnosed by enzyme-linked immunosorbent assay (ELISA) method according to the National AIDS Control Organization (NACO) guidelines were included. Complete hematologic profile was recorded. Relationship between CD4 counts and various hematologic parameters was analyzed. Descriptive statistics were applied. Association between two attributes was calculated by chi-square test and P value less than 0.05 was considered statistically significant.

Results: The most common hematologic abnormality was anemia, seen in 85% (n=85) of the patients. Normocytic normochromic anemia was seen in 57 patients,which was the most common type. A strong association between anemia and CD4 counts was observed. The hemoglobin, red blood cell count and hematocrit also showed corresponding low values with decreasing CD4 counts. Thrombocytopenia was the second common abnormality and had no significant relationship with CD4 counts.

Conclusion: HIV affects virtually all organ systems, with well-known abnormalities related to hematopoietic system. Anemia being the most common abnormality has strong correlation with CD4 counts and therefore could be used to predict the development of a more advanced disease.

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Introduction

Acquired immunodeficiency syndrome (AIDS) is a caused by the HIV virus and characterized by severe immunosuppressionthat leads to opportunistic infections, neoplasms and neurological manifestations. [1,2]

It is a major health challenge in the modern world causing damage in the resource poor south-east Asian countries. It involves almost all the systems in human body. [3,4]More people than ever are living with HIV, largely due to greater access to treatment. It is fast cbecoming a major health hazard in the Indian subcontinent, with an estimated 3.7 million persons being infected with HIV. [5]

Hematologic abnormalities are among the most common complications of HIV. These involve all the lineages of the blood cells. Anemia is the most common hematologic abnormality affecting 60% to 80% of patients in the late stage disease with high viral load. [1,6]Neutropenia is caused by inadequate production and thrombocytopenia by immune-mediated destruction of platelets in addition to inadequate production. [1,7]As the infection progresses, there is development of pancytopenia. [3]

HIV associated hematologic expressions seem to be dependent on the level of viral replication, as these abnormalities are severe in AIDS patients with high viraemia and decreased CD4 counts. [1]There are a few studies on haematological changes in HIV and a very few have correlated results with CD4 count. [8]In the present study, the haematologic changes have been studied and correlated with CD4 cell counts to highlight these manifestations in disease progression.

Materials and Methods

One hundred patients, seropositive for HIV by ELISAwere included in the study. Ethical clearance from the institutional ethical committee was obtained.Demographic and clinical informationwere recorded. Complete hemogramusing automated cell counter –Sysmex XN-1000 was done and various parameters including hemoglobin, total leucocyte count (TLC), Absolute neutrophil count (ANC) and absolute lymphocyte count (ALC) differential leucocyte count, platelet count, red blood cell indices, red cell distribution width (RDW), hematocrit (HCT) and reticulocyte count were noted.Prothrombin Time (PT) and activated partial thromboplastin time (aPTT) were done using semi automaticcoagulometeranalyser CA 50 SYSMEX.CD4 count was available in seventy patients. CD4 lymphocyte count was done in BD FACS Caliburflowcytometer.

Anemia was defined as hemoglobin <13 g/dl (Men) and <12 g/dl (women, non-pregnant). Leucopenia was defined as TLC of less than 4000 cells/mm³. Neutropenia was defined as ANC of <1000 cells/mm³. Lymphopenia was considered when ALC was <800 cells/mm³ Thrombocytopenia was defined as total platelet count $< 150 \times 10^3/mm^3$ [1].

Statistical Analysis

Descriptive statistics were expressed as Mean ± Standard Deviation and results on categorical measurements as numbers (%). Comparison between data was done by chi-square test. A P-value <0.05 was taken as statistically significant. Statistical software namely SPSS 15.1, Stata 8.0, MedCalc 9.0.1 and Systat 11.0 was used for analysis of data.Microsoft word and Excel were used to generate graphs and tables.

Results

Demographic Data: The youngest patient was seven years old and the oldest was 80 years old and the mean age was 39.8 ±10.7 years. Most patients (45%) were in the age group of 31-40 years. Sixty five patients were males and thirty five were females with a male to female ratio of 1.9:1.

Clinical Presentation and CD4 Counts: Seventy eight patients were symptomatic with fever (55%), generalized weakness (35%), diarrhea (20%), loss of weight /appetite (23%), and productive cough (26%). Patients were divided into three categories depending on CD4 counts; category 1 –less than 200 cells /μL,category 2 - 200 to 499cells/μL and category 3 –more than 500cells/μL [8].Most patients belonged to category 1 (51.4%).Twenty one patients were on antiretroviral therapy. However, majority of them were taking irregular treatment.

Anemia: Most of the patients presented with anemia (85%) and was more common in symptomatic patients, accounting for 83% (n=71). Anemia was graded based on hemoglobin level into severe anemia with hemoglobin <8 gm/dl and mild to moderate anemia with hemoglobin >8gm/dl. Severe anemia was seen in 20% (n=17)) and mild to moderate anemia in 80% (n=68) of the cases. In relation with CD4 counts, 53% (n=32) of category-1 patients had anemia while among category-2 and category-3 patients, anemia was seen in 33% (n=24) and 14 % (n=10) patients respectively, which was statistically significant (P<0.005). The mean hemoglobin, HCT and RBC countsalso showed statistically significant Pvalue when compared with CD4 counts (Table1). The commonest type of anemia was normocytic normochromic anemia (NNA), which accounted to 67.1% (n= 57). Microcytic hypochromic anemia (MHA) was seen in 23.5% patients (n =20) and macrocytic anemia (MA) in six patients.

Leukopenia: Leukopenia was seen in 20% (n=20) of the patients. Neutropenia was seen in three patients. Twenty

three patients had lymphocytopenia. TLC, ANC and ALC also showed corresponding low values with a decrease in CD4 count. However the *P* value of these parameters was not statistically significant (Table 1).

Thrombocytopenia: Thirty two patients had thrombocytopenia. There was a reduction in the platelet count with a decrease in CD4 count. However, the *P* value was not statistically significant (Table/figure1). Eight patients had pancytopenia. Coagulation profile did not show significant changes.

Discussion

The most common source of HIV disease throughout the world is HIV-1 which was first identified in 1981 in West Africa. The first AIDS case in India was detected in Chennai and since then HIV infection has been reported in all the states and union territories. [3,9]The spread of HIV in India has been irregular with more severe epidemics being reported in the southern India and the north-east. 3Worldwide, the phenomenon of HIV/AIDS is best viewed as a pandemic affecting nearly all the countries of the world. [10]

Hematological abnormalities are among the most common complications of HIV. These involve all the lineages of blood cells. [11] HIV associated haematological abnormalities appear to be dependent on the level of virus replication, as these abnormalities are severe in late-stage AIDS patients with high viremia. The mechanism underlying these abnormalities is still equivocal. A specific diagnosis of the cause and mechanism must be required because specific treatment may be needed for its correction.[11]

Anemia is the most common cytopenia in HIV-infected individuals, occurring in 10-20 % of patients at initial presentation and diagnosed in approximately 70 -80 % of patients over the course of disease. The incidence is strongly related with the progression of the disease and is common in the symptomatic group. [1,12,13,14,15,16,17]

In the present study, severe anemia was observed in 20% (n=17) as compared to 7%, 18.5% and 33% in various studies. [1,6,12]Thirty six (53%) patients were anemic with a CD4 count of <200 cells/μL. [1Attili et al[7] found a strong correlation between CD4 count and severity of anemia in their hospital- based cohort study. The cumulative incidence and severity of anemia was highest among patients who had CD4 count of < 200 cells/ μL and was lowest with CD4 count of >500 cells/ μL. Sullivan et al, in their study reported that the incidence of anemia was strongly and dependably associated with the progression of HIV disease as measured by diagnosis of AIDS defining opportunistic illness and measurement of CD4 count < 200 cells/μl. [19]

In the present study, the presence of anemia correlated with disease progression as most of the patients with anemia belonged to category 1 (CD4 counts of <200cell/mm³). This association is most likely explained by the increasing viral burden as HIV disease progresses, which leads to cytokine-mediated myelosuppression and anemia. [19]Inflammatory cytokines released by lymphocytes such as tumour necrosis factor (TNF), interleukin-1 (IL-1) and interferon gamma play an important role in the pathogenesis of anaemia. These cytokines inhibit erythropoiesis in vitro. [20] TNF levels were found to be consistently elevated in HIV infection and this condition is correlated with viral load.21Studies also clearly indicate that anemia does affect the survival of HIV infected patients. [1,3,7,18,22]

The HGB, RBC and HCT levels also showed correspondingly low values with decrease in CD4 counts. The *P* value was statistically significant. The results of a study by Mocroftet al[23]showed that hemoglobin levels provided prognostic information independent of that provided by CD4 count. The overall frequency of anemia in this study group was comparable with other studies (Table2). [24,25,26,27,8,28]

Risk factors currently associated withanemia in HIV infection include clinical AIDS, CD4 cell count of <200 cells/μL, women, plasma viral load, black race, zidovudine use, lower body mass index, increasing age, bacterial pneumonia, oral candidiasis and fever. [29]

The increased frequency of normocytic normochromic anemia (67.1%) in the present study was in accordance with some studies and discordant with others(Table 3). [1,6,25,30,8,28] In the present study, we did not find any patient with hemolytic anemia. Macrocytosis has been well described in patients undergoing antiretroviral therapy with zidovudine. [12,18,20] In the present study group, the six patients with macrocytic anemia were on irregular ART, which could explain anemia in this group.

Leukopenia typically involves granulocytes and lymphocytes, although monocytopenia has also been reported in patients with the diagnosis of AIDS. Neutropenia of less than 1000 cells/μL is reported in approximately 10% of patients with early, asymptomatic HIV infection and in more than 50% of individuals with advanced HIV related immunodeficiency.[3,13,20,29]Neutropenia is often caused or exacerbated by concomitant myelosuppressive drugs. Adverse drug reactions and their complications can also cause neutropenia in patients with HIV/AIDS. [1] Various studies have reported neutropenia in 13% to 44% of cases with progression of disease from HIV to AIDS. [7] In the present study, only three patients were detected to have neutropenia. Dikshith et al [1] did not identify any case

Table 1: Correlation of haematological parameters with CD4 counts.

CD 4 count (cells/µL)	<200	200-499	500+	*P* value
HGB (mean±SD) g/dl	9.6±2.2	9.5±2.2	12.07±1.5	0.007**
RBC count (Mean±SD) millions/µL	3.3±0.7	3.5±0.8	4.3±0.6	0.003**
HCT (Mean ± SD) %	30.63±6.04	31.74±6.6	36.65±4.8	0.02*
TLC (cells/µL)	6358±3774	6237±3028	8870±4419	0.126
ANC(cells/µL)	4897±3505	4737±3107	6184±4040	0.51
ALC(cells/µL)	1383±694	1554±1059	1953±725	0.16
Platelets L/ mm^3(Mean±SD)	1.83±1.17	1.89±0.98	2.17±1.41	0.7

(significant **P** value)*

Table 2: Comparison of number of anaemia cases in the present study with other studies.

Authors	No of anaemia cases	Total cases	Percentage
Karcher et al	175	197	89 %
Tripathi et al	61	74	82.4 %
Sitalakshmi et al	27	42	64.2 %
Kaloutsi et al	34	40	85 %
Parinitha et al	210	250	84 %
Rahman et al	103	204	50.5%
Present study	85	100	85%

Table3: Comparison of morphological patterns of anemia in the present study with other studies.

Patterns of blood picture	Tripathi et al		Khandekar et al		Parinitha et al		Rahman et al		Present study	
	Number	%	Number	%	Number	%	Number	%	Number	%
Normocytic normochromic anaemia	54	72.9	68	48.57	101	40.4	28	13.7	57	67.1
Microcytic hypochromic anaemia	4	5.4	15	10.71	18	7.2	10	4.9	20	23.5
Macrocytic anemia	3	4.1	32	22.86	15	6	30	14	6	7.1

of neutropenia which differed from a study by Attili et al in which 22.7% of patients had neutropenia. [7]

The correlation between the level of ANC and the need for hospitalization for bacterial infections has also been proven in some trials. [31]Thus, it could be important to recognize patients with neutropenia, who are at increased risk of developing these infections. HIV infection can directly result in lymphopenia as the infection evolves, leading to a decrease in CD4+ lymphocytes and is one of the most important prognostic indicators for the risk of developing opportunistic infections. [32]

In the present study, lymphopenia was seen in 23/100 (23%) of patients. There has been a variation in the frequency of lymphopenia reported in different studies which includes 28.9% (59/204), 65.2%(163/250), 70% (14/20) and in 25.6% (19/74) of the cases. [28,8,33,25] A significant association of absolute lymphocyte count with CD4 cell counts was observed in some studies. [8,28]In

the present study, lymphopenia was defined as when the patients had an absolute lymphocyte count of <800cells/mm^3. However, the cut-offs in the other studies was in the range of <1000-1500 cells/mm^3. This could probably explain this variability.

The TLC, ANC and ALC did not show a statistically significant association with CD4 count, although the counts were low with decreasing CD4 counts. The sample size and the cut-off of lymphopenia might elucidate this.

Thrombocytopeniais reasonably common during the course of HIV infection, occurring in approximately 40% of patients. It serves as the first symptom or sign of infection in approximately 10% of individuals. [34]Presence of thrombocytopenia is independent of the disease progression. The mechanism of thrombocytopenia in HIV infection is mainly due to ineffective platelet production and at the same time increased platelet destruction. [35]

Prevalence of thrombocytopenia is reported to be higher among persons with AIDS, homosexuals, older persons and injecting drug users.[36]

In the present study,thrombocytopenia was seen in thirty two patients.Various studies have reported thrombocytopenia in 18% (45/250), 13% (65/500), 13% (121/925) and 3.4% (7/204) of the cases.[8,37,38,28] However, Karcher et al [24] reported thrombocytopenia in 88/196 (45%) cases, which was in accordance with the present study. It was the most frequent cytopenia observed by Kasturi et al and a feature seen in advanced disease. [12]However, in the present study, no significant relationship with CD4 counts was observed,endorsing the data of the previous studies. [1,7]

Our study reported pancytopenia in eight patients.In the present study, coagulation abnormalities were not seen. Though the increased risk of venous thrombosis is known in HIV infection, coagulation abnormalities are not well established. In a CDC study, it was found that overall incidence of thrombotic episode was 2.6 per 1000 HIV-infected persons. [7]

Conclusion

Hematologic abnormalities are the common manifestations in patients with HIV/AIDS. All the cell lines are affected by HIV, resulting in anemia, thrombocytopenia and leukopenia. The recent surge of HIV infection has led to an increase in the incidence of hematologic abnormalities. Anemia is the most common hematologic abnormality which is more prevalent among patients with declining CD4 count and is strongly associated with progression of the disease. Hence, it is prudent to investigate and find the cause of anemia for instituting specific treatment. The severity of other peripheral cytopenias is related to the disease burden and has got significant impact on clinical outcome. Early detection, exact cause and appropriate treatment of these abnormalities will reduce morbidity and mortality in HIV/AIDS patients.

The spectrum of these problems has been recognized in certain parts of the world. The study of nature of these hematological abnormalities is therefore relevant in a tertiary care centre in a south Indian population.

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