



A Study of the Hematological Profile of Human Immunodeficiency Virus Positive Patients in Coastal South Indian Region

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Introduction: In India, approximately 6 million populations are affected by human immunodeficiency virus (HIV). Anemia and leukopenia, especially thrombocytopenia is seen commonly in HIV infections. Low CD4+ count and increased viral load are some of the factors associated with increased risk of thrombocytopenia. We analyzed the hematological profile in a group of 150 HIV infected patients. **Materials and Methods:** A retrospective and prospective study of medical records of 150 HIV positive patients at Clinical Pathology laboratory at our institution was done between August 1st and October 15th, 2011 using nonrandom sampling. Hemoglobin (Hb), hematocrit, red cell indices, total leukocyte and differential count, CD4+ and platelet count were noted. **Results:** Of the 150 patients, 40 (26.67%) were below age 10 and 98 (65.33%) in 21-50 years age group. Eighty-six (57.33%) were females. Hundred patients had anemia (Hb <12 g/dl) of which 58% were microcytic hypochromic (MCHC). Eighteen patients had leukopenia along with anemia. Total number of patients with low CD4 count (<200/ μ L) was 32 (21.33%) and all had hematological abnormalities, mostly anemias with few leukopenia and thrombocytopenias. All patients with pancytopenia had low CD4+ counts. Total number of patients with thrombocytopenia (<1.5 lacs/dl) was 20 (13.33%). Four patients (2.67%) had pancytopenia. **Conclusions:** MCHC anemia is the most common morphological variant of anemia. Leukopenia was found to be consistently associated with anemia. Thus, anemia and to a greater extent leukopenia are bad prognostic indicators of disease. Pancytopenia may herald a low CD4+ count.

Key words: Human immunodeficiency virus, hematological profile, anemia, leukopenia, thrombocytopenia, pancytopenia

INTRODUCTION

According to the World Health Organization, human immunodeficiency virus (HIV) has infected 33.2 million people; thus, aptly termed “global pandemic.”¹ In India, while more than 1.4 million suffer from full-blown acquired immunodeficiency syndrome (AIDS), approximately 6 million populations are already affected by this disease.²

Hematological manifestations of varied types occur in HIV-seropositive individuals at all stages of HIV infection. Anemia, thrombocytopenia, and neutropenia are common manifestations in patients with HIV infection that become more frequent and severe with progression from the asymptomatic state to AIDS.

An assessment of hematological profile helps the physician in instituting proper treatment. To ensure better management

protocol for patients infected with HIV and improving their quality of life, it is necessary to look into their hematological profile, which forms the preliminary investigation in most ailing people presenting to health care professionals.

The aim of the study was to analyze the hematological profile in a group of 150 HIV-infected patients presenting at a tertiary care center in Karnataka. The primary objective was to assess the extent of anemia as mild (10-12 g/dl), moderate (8-10 g/dl) and severe (<8 g/dl). The other objectives were to:

1. Evaluate the prevalence of leukopenia in the HIV seropositive individuals.
2. Estimate the frequency of thrombocytopenia among HIV-infected patient.
3. Review the type of anemia and morphologically classify as normocytic normochromic (NCNC), microcytic hypochromic (MCHC) or macrocytic, or hemolytic.
4. Evaluate the degree of neutropenia and lymphopenia.
5. Correlate with the CD4+ counts wherever feasible.

MATERIALS AND METHODS

It was a retrospective and prospective study based on medical records of HIV-positive patients received at Clinical

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Pathological Laboratory of our institution between August 1st and October 15th, 2011. Nonrandom sampling was used. The records are analyzed for hemoglobin (Hb), hematocrit, red cell indices, total leukocyte count (TLC) and differential count, CD4+count, platelet count. These hematological parameters were analyzed from blood samples in Beckman-Coulter LH780 automatic hematology analyzer. The quality checks of the instrument are performed according to the manufacturer's instructions with two-level controls daily. In addition, according to the laboratory policy, the instrument is also subjected to external quality assurance programs.

RESULTS

Of the 150 patients studied, 86 (57.33%) were women. The female to male ratio is 1.34:1. Forty (26.67%) are below age 10, 98 (65.33%) are within the age group of 21-50 years. Mean age was 27.1. Mean age among males is 27.92 and among females is 27.75 years. The age and the sex profile are shown in Figure 1.

Red blood cell profile

Hundred patients had anemia (Hb <12 g/dl). Mean Hb was found to be 11.25 g/dl. About 79% of female patients and 50% of male patients were found to be anemic. The grading of anemia is shown in Figure 2.

Morphologically, 58 (38.7%), 38 (25.3%) and 4 (2.7%) were MCHC, NCNC and macrocytic, respectively.

Of the 100 anemic patients, 70 had anemia alone. Anemia with leukopenia was found in 14, with thrombocytopenia observed in 12 and pancytopenia, 4 patients.

White blood cell profile

Mean total leukocyte count was 6874/ μ L. Of the 18 leukopenic (TLC <4000/ μ L) patients, 8 had absolute

lymphopenia (<1000/ μ L) and 6 had absolute neutropenia (<1500/ μ L) and 4 had both. All the 18 patients suffered from anemia.

CD4+ profile

Total number of patients with low CD4 count (<200 cells/ μ L) was 32. Mean CD4 count was 375.6/ μ L. The lowest count was 57/ μ L; highest was 1200/ μ L. Of the 32 patients, 22 suffered from anemia, three from thrombocytopenia and three, leukopenia out of which, three had absolute lymphopenia, and one had both absolute lymphopenia and neutropenia. Pancytopenia was observed in 4 patients.

Platelet profile

Total number of patients with thrombocytopenia (platelet count <1.5 $\times 10^5$ /dl) was 20 of which 8 were associated with low CD4 counts. Mean platelet count was 2.35 $\times 10^5$ / μ L. Isolated thrombocytopenia was noticed in 6 patients, 10 (50%) had associated anemia and 4 (20%) had pancytopenia.

DISCUSSION

Anemia is common over the course of the disease seen in 70-80% of the patients.³ The multifactorial causes include HIV infection itself (most common) bone marrow suppression by anti-retroviral drugs, opportunistic infection in AIDS by atypical tuberculosis, mycobacterium tuberculosis, parvovirus B19, cytomegalovirus, etc. Zidovudine is associated with macrocytic anemia. True autoimmune hemolytic anemia is rare.¹ Anemia leads to decreased survival (150% more relative risk of death with baseline CD4+ count <200 cells/ μ L in those with anemia than in those without), rapid disease progression and impaired quality of life.³

Neutropenia develops in both early, asymptomatic HIV infection and advanced AIDS in about 10 and 50% patients respectively.³ HIV infection by itself, HIV-related myelodysplasia, drug therapy for HIV, malignancies, and the various opportunistic infections offer the multitude of

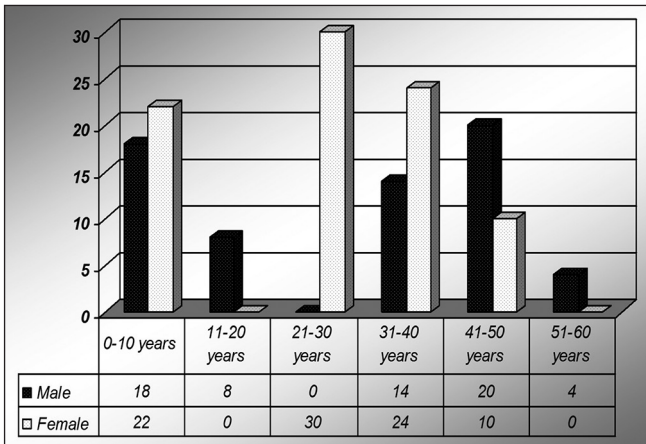


Figure 1: Age and sex profile of human immunodeficiency virus/acquired immunodeficiency syndrome patients

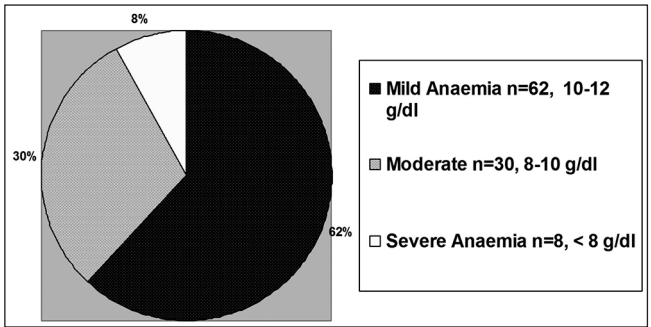


Figure 2: Grading of anemia based on severity (n = 100)

Hematological profile of HIV positive patients

reasons for this disorder. The classic hallmark of the disease, lymphopenia involves primarily the T-helper cells that deteriorate with disease progression. Lymphopenia is a result of the direct cytopathic effects of the virus.³

Among HIV patients, during the course of the disease and as the first sign of infection, thrombocytopenia is observed in approximately 40 and 10%, respectively. Low CD4⁺ count and increased viral load are some of the factors associated with increased risk of thrombocytopenia. Antiplatelet antibodies, zidovudine therapy, and direct infection of megakaryocytes by HIV are some of the mechanisms implicated.³⁻⁵

The female to male preponderance seen in our study was in contrast with other studies.⁶⁻⁹ This could be because of the small sample size or due to the increased health awareness among women in this district. Majority of the 150 patients (42.67%) were in the age group of 21-50 years as was observed in the study by Ogun *et al.*⁸. However, mean age among patients in our study was lower as compared to other studies.⁷⁻⁹ High occurrence of HIV infection, 40 cases (26.67%) in below 10 age group has been observed. This reflects the need for adoption of effective procedures to prevent parent to child transmission.

Anemia (Hb <12 g/dl) was seen in 100 (66.67%) of our patients. This is less as compared to other studies.⁶⁻¹⁰ This is in spite of the fact that the cut-off level for anemia in our study was higher as compared to other studies where the cut-off level was 10 g/dl.⁶⁻⁹ Attili *et al.* considered Hb below 12 g/dl to be anemic.² High association of anemia may be due to preexisting iron deficiency, defects in iron metabolism, anti-retroviral therapy, opportunistic infections and advanced stage of the disease. Hb <10 g/dl was observed in 57 (38%) of the anemic patients, of whom only 8 (5.3%) had severe anemia (Hb <8 g/dl). This low occurrence of severe anemia is comparable to other studies.^{7,9} Mean Hb in our study was 11.25 g/dl, similar to study by Dikshit *et al.*⁹ Prevalence of anemia among female HIV patients (68%) was appreciably more than their male counterpart (32%).

Of the anemic patients, MCHC dominated the picture with 58 followed by NCNC with 38 after which came the macrocytic variant with 4%. The predominance of MCHC anemia is highly significant ($\chi^2 = 22.4266$, $P < 0.0001$) in our study. The preponderance of the MCHC overwhelmed that of the other studies.^{6,7,9} Coexistent nutritional deficiency could be responsible. Macrocytic anemia might be attributed to Vitamin B12 deficiency or zidovudine therapy.¹

Anemia was found to be consistently associated with leukopenia — all patients with leukopenia had anemia. Thus, it is a tempting assumption that anemia appears first and then leukopenia takes center stage, may be at a later stage of the disease. Since leukopenia predisposes the individual to

secondary infections, it seriously presages a compromised quality of life that tells upon overall longevity. Thus, the hypothesis that anemia in HIV-infected patients, if the iron deficiency can be excluded is a bad prognostic indicator and leukopenia seriously augments the risk may have discovered some foundation on the present study. Anemia could also be deemed as a forerunner of other hematological cytopenias in an HIV-infected patient. Anemia was the most common hematological abnormality in patients with the low CD4⁺ count. This association is also seen in other studies.^{9,11} This may be due to the institution of zidovudine therapy, more opportunistic infections or advanced stage of disease associated with low CD4⁺ counts.

Mean total leukocyte count in our study was 6874 cells/ μ L, similar to the study by Dikshit *et al.*⁹ Similar to other studies, the rate of leukopenia in the present study was lower compared to anemia⁷⁻¹¹ but in contrast, leukopenia and thus lymphopenia ($n = 10$, 6.67%) and neutropenia ($n = 8$, 5.33%) was much lower compared to most studies.^{2,4,6,8,10} Ten leukopenic patients were associated with low CD4⁺ counts of which 8 were lymphopenic and two, neutropenic in the present study. Association of lymphopenia with low CD4⁺ count was also seen by Amballi *et al.*⁸ As a disparity, strong association of neutropenia with low CD4⁺ count was not observed.^{2,6,12}

Patients with thrombocytopenia in our study ($n = 20$, 13.33%) were more compared to that of Attili *et al.*² Of these, since only 8 showed a low CD4⁺ count, thus positive correlation between low CD4⁺ count and platelet count^{2,9} could not be established.

A notable observation in our study was the association of all cases of pancytopenia with low CD4⁺ counts. Since only four pancytopenics were seen, a significant correlation could not be proven. It may not be presumptuous to surmise that a low CD4⁺ count predisposes to pancytopenia thus alluding to pancytopenia being a harbinger of the low CD4⁺ count. In cases, where estimating CD4⁺ counts at regular intervals may be financially constricting, complete blood counts by a good hematology analyzer may to some extent suffice the deficit.

One of the limitations of this study has been that the sample size was small compared to the prevalence of the disease. This study can be considered only as a pilot study and should be extrapolated to a bigger study with case and control group selected within the sample, for example - those with low and high CD4⁺ counts.

SUMMARY AND CONCLUSION

HIV infection is riddled with hematologic alterations and may be due to the direct effects of HIV, secondary infection, neoplasm, or adverse effects of therapy.

Anemia was observed to be the most common hematological abnormality in this setting ($n = 100$, 66.66%). Of the morphological variants, a higher percentage of hypochromic microcytic anemia ($n = 58$, 38.7%) was observed. This is extremely significant in the Indian scenario ($\chi^2 = 22.4266$, $P < 0.0001$) and emphasizes the need to estimate serum ferritin to detect concomitant iron deficiency and thereby remedy the same, preferably parenterally. All patients with leukopenia suffered from anemia. Thus, anemia to a lesser degree and leukopenia seriously are bad prognostic indicators representing the advancement of the disease process in HIV infection. Anemia as a hematological abnormality may also precede other hematological cytopenias. Thus, in the anemic patient, physicians have to be vigilant about monitoring blood counts regularly to detect and implement proper measures to counteract the ill-effects of bi-or pancytopenia. Relatively, lower prevalence of leukopenia was detected in our study as compared to other studies. All cases of pancytopenia had low CD4+ counts. Thus, a relatively cost-effective test like complete blood count that detects pancytopenia may serve as a forerunner of low CD4+ counts. A high rate of HIV disease in childhood is a cause for concern.

Disease progression may be predicted and prognosticated especially in children by the severity of anemia. Leukopenia and especially neutropenia incline the patient to bacterial infections. Hence, in the HIV-infected patient, these parameters may alert to institute prophylaxis against opportunistic infections in time. The usefulness of this study may be gauged by the well-known fact that anemia, absolute neutropenia and absolute lymphopenia are good clinical indicators of the underlying immune status along with predictor of the clinical stage of HIV/AIDS and thus highlights the importance of a larger and a better organized study.

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