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Original Research Article

Haematological profile of human immunodeficiency virus infected patients

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ABSTRACT

Background: 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. The aim of the study was to study the hematological changes that occur in HIV infected patients who attend the Institute of Venereology, before starting HAART.
Methods: This cross-sectional study was conducted in the Institute of Venereology, Madras Medical College/Rajiv Gandhi Government General Hospital, Chennai in 100 treatment-naive HIV infected patients. Detailed history and clinical examination was done. Blood samples were collected. Complete blood count, CD4 count, prothrombin time, activated plasma thromboplastin time, peripheral smear etc., were done. Results were collected and analysed.
Results: Out of 100 patients, 56% were males and 43% females and one transgender. Anaemia was detected in 72%patients. 73.5% males and 76.2% females with CD4 count <350/µl were anemic. The commonest anaemia was normochromic normocytic, seen in 65% patients. 7 male and 7 female patients had leukopenia. 81.25%patients with lymphocytopenia had CD4 count <350/µl. 12% males and 4% females had neutropenia. 17% had neutrophilia. Patients in WHO stage I did not have neutropenia. 23% patients had thrombocytopenia. 47% patients with thrombocytopenia were in stage IV. Maximum number of patients with normal platelet count was in stage I.
Conclusions: Haematological abnormalities are a common occurrence during the course of HIV infection. Identifying and treating the haematological changes have great impact on both the morbidity and mortality of HIV infected patients.

Keywords: Human immunodeficiency virus, Hematological profile, Anemia, Leukopenia, Thrombocytopenia, Pancytopenia

INTRODUCTION

HIV infection is a major public health problem. Throughout the world, 34 million people are living with HIV/AIDS. Nearly 20 lakh HIV infected people live in India. HIV/AIDS is a multisystem disease. Hematological abnormalities are one of the most common complications. Almost all cell lineage in the bone marrow is affected manifested by anemia, leukopenia, neutropenia, thrombocytopenia, coagulopathy, etc.[1]

Identification of the hematological changes helps in diagnosing opportunistic infections, malignancy, disease progression. Correcting hematological changes reduce the

complications and improve the quality of life.[2] Impaired hematopoiesis, immune-mediated cytopenias, and altered coagulation mechanisms occur. They are due to direct action of HIV, opportunistic infections, malignancies or treatment.[3] Anemia is the most frequent hematological abnormality. Prevalence of anemia is 63% to 95%. Anemia occurs due to defect in the metabolism and utilization of iron, drugs, vitamin B12 deficiency, opportunistic infections. Anemia of chronic disease is an important cause of HIV infection. The presence of anemia carries an increased risk of death and is independent of the CD4 count or viral load.[4] Anemia correction is associated with decreased mortality and improved quality of life. Small increases in the level of hemoglobin (2 gm/dl) can cause improvement in the quality of life.

Earlier studies show that thrombocytopenia occurs in 40% HIV infected patients. Megakaryocyte itself is a target for HIV. Prevalence of thrombocytopenia is higher in AIDS patients, older persons, homosexuals, and injection drug users. Causes of thrombocytopenia include direct infection of megakaryocytes, bone marrow infiltration by opportunistic infections, neoplasms, thrombotic thrombocytopenic purpura, and drug-induced myelosuppression. Neutropenia is seen in 10% of patients in the early stages and 50% of patients in the advanced stages. Neutropenia is defined as absolute neutrophil count <1000 cells per microliter. It is inversely proportional to the CD4 count. A shift to the left in the granulocyte series is universal. Thrombosis occurs in nearly 2% of HIV infected patients. Prolonged Prothrombin and activated plasma thromboplastin time are seen in patients with CD4 count < 200/µl.[5]

Rarely haemophagocytic syndrome can occur. Haemophagocytosis is characterized by the proliferation of histiocytes and phagocytosis of marrow blood cell precursors. Peripheral blood smear in HIV infected patients shows anisocytosis, poikilocytosis, Rouleaux formation, neutropenia, lymphopenia, and monocytopenia. A shift to the left is seen in the granulocyte series. In advanced stages, vacuolated monocytes can be seen. Neutrophils may reveal dysplastic changes. They may show hypersegmentation or Pelger-Huet forms. Neutrophils can also be large and show prominent granulation. Atypical lymphocytes are also seen. In HIV associated immune thrombocytopenic purpura, large platelets are seen.[6]

Aim

- To study the hematological changes that occur in HIV infected patients who attend the Institute of Venereology, before starting HAART.
- To correlate the hematological indices with the WHO clinical staging of HIV/AIDS.
- To associate the hematological indices with the immune status of the patients as reflected by the CD4 count.

METHODS

This cross-sectional study was conducted from September 2012 to November 2013 in the Institute of Venereology, Madras Medical College/Rajiv Gandhi Government General Hospital, Chennai in 100 treatment-naive HIV infected patients. Inclusion Criteria: Patients found seropositive for HIV infection, strategy 3 is employed for diagnosing HIV infection. Exclusion criteria: Pregnant women, patients already on HAART, patients unwilling to participate. At recruitment, blood samples were collected in a plain vial under strict aseptic precautions for HIV testing. Patients found seropositive for HIV infection strategy 3 are taken for the study. The patients are subjected to detailed history taking and complete clinical examination. Symptoms due to opportunistic infections, anaemia, etc., are elicited in the history. Detailed sexual history, personal history, and history regarding any chronic illness are elicited. General examination, system examination, complete genital and dermatological examination was undertaken. WHO clinical staging was done. Blood samples were collected under strict aseptic precautions, and the following tests were done. Absolute CD4 lymphocyte count was determined by flow cytometry using Partech CD4 machine. Diagnosis of syphilis was established by VDRL. Complete blood count was done using SYSMEX cell counter. Prothrombin time and APTT was estimated using SYSMEX coagulation analyser. Peripheral smear was taken and stained by Leishman's stain. Liver function tests and renal function tests were done. X-ray chest and sputum AFB was done to rule out tuberculosis. Ultrasound abdomen was done. Other investigations like Tzanck smear, wet mount, KOH mount, Gram staining, dark field microscopy, gonococcal culture and sensitivity, and TPHA were done for patients with sexually transmitted infections. Data were analyzed using SPSS 16.

RESULTS

Out of the total 100 patients, 56 patients (56%) were males. There were 43 females (43%) and one transgender (1%). In the study, majority (78 patients) were in the 26–50 years age group (78%). There were only 2 patients in the 0–12 years age group. 92 patients (92%) were heterosexuals. 4 patients acquired HIV infection from the parents. 2 patients (2%) patients were bisexuals and 2 patients were homosexuals.

Majority of the patients (33%) were in stage II of WHO clinical staging followed by 29% patients in stage III. 21% patients were in stage I. Only 17% patients were in stage IV.

14% patients had CD4 count <100 cells/µl, 16% had CD4 count in the range of 101–200 cells/µl, 17% in the range 201–300 cells/µl, 19% had CD4 counts in the range of 301–400 cells/µl, 12% in the range of 401–500 cells/µl and 22% patients had CD4 count above 500 cells/µl. 15%

patients had various sexually transmitted infections. 4 patients had syphilis and one t had gonococcal urethritis. One patient presented with HIV wasting syndrome. 20% patients were asymptomatic at the time of presentation. Fatigue was observed in 16% patients.

Table 1: Hemoglobin level.

HB level gm/dl	Sex			
	Males	Females	Trans-gender	Total
<6	2 (50.0)	2 (50.0)	0 (0)	4 (4)
6.1-9	11 (42.3)	15 (57.7)	0 (0)	26 (26)
9.1-13	25 (51)	24 (49)	0 (0)	49 (49)
>13	18 (85.7)	2 (9.5)	1 (4.8)	21 (21)
Total	56 (56)	43 (43)	1 (1.0)	100 (100)

4% patients had haemoglobin level <6 gm/dl and 26% had haemoglobin level in the range 6.1–9 gm/dl. 49 patients had haemoglobin level in the range 9.1–13 gm/dl. 21 patients had haemoglobin level >13 gm/dl. 6.6% patients with CD4 count <200 cells/µl had haemoglobin levels <6 gm/dl, whereas only 2.8% patients with CD4 count >200 cells/µl had haemoglobin <6 gm/dl. Only 6.6% of patients with CD4 count <200 cells/µl had haemoglobin levels >13 gm/dl. But 27.1% patients with CD4 count >200 cells/µl had haemoglobin >13 gm/dl. This shows a direct correlation between haemoglobin level and the CD4 count.

Majority of patients in stage I (50%) had hemoglobin level in the range of 9.1 gm/dl to 13 gm/dl. 35% had hemoglobin >13 gm/dl. Only 5% patients had hemoglobin level <6 gm/dl.

In stage II, 41.2% of patients had hemoglobin in the range 9.1 gm/dl to 13 gm/dl. In this group 35.3% had hemoglobin level >13 gm/dl. 20.6% had hemoglobin level in the range 6.1 gm/dl to 9 gm/dl. Only 2.9% had hemoglobin level in the range <6 gm/dl.

In stage III, 6.9% patients had hemoglobin level >13 gm/dl. In stage IV, none of the patients had hemoglobin more than 13 gm/dl. Majority of the patients (58.8%) had hemoglobin in the range 6.1 gm/dl to 9 gm/dl. The Chi-square value is 24.012. P value is 0.004 ($p<0.05$). This shows WHO staging and hemoglobin level has a significant correlation.

Table 2: Anaemia and who staging.

WHO staging	Males (n=56)		WHO staging	Females (n=43)	
	Number	Percentage (%)		Number	Percentage (%)
Stage I (n=7)	2	28.6	StageI (n=12)	9	75
Stage II (n=23)	12	52.2	Stage II (n=11)	8	72.7
Stage III (n=18)	16	88.9	Stage III (n=11)	9	81.8
Stage IV (n=8)	8	100	Stage IV (n=9)	8	88.9

Table 3: Leucocyte count distribution.

Leukocyte count Cells/mm^3	Males (n=56)		Females (n=43)		Transgender (n=1)	
	Number	Percentage (%)	Number	Percentage (%)	Number	Percentage (%)
<4000 (n=14)	7	7	7	7	0	0
4001-11000 (n=82)	48	48	33	33	1	1
>11000 (n=4)	1	1	3	3	0	0

28.6% males in stage I was anemic. 52.2% of males in stage II and 88.9% in stage III were anemic. All males in stage IV were anemic. The Chi-square value is 14.987. P value is 0.002 ($p<0.05$). The level of anemia in males has a significant relationship with WHO staging.

Among female patients, anemia was more prevalent in stage IV than any other stage (88.9%). 73.5% of males with CD4 count <350 cells/µl were anemic. Only 58.3% males with CD4 count >500 cells/µl were anemic. Chi-square value is1.284 and the P value is 0.526 ($p>0.05$). So the relationship between the level of anemia in males and CD4 count is statistically insignificant.

7 males had total leucocyte <4000 cells/cumm. 48 male patients had total leucocyte count in the range 4001 to 11000 cells/cumm, and one male patient had leucocyte count >11000 cells/cumm. 7 females had total leucocyte count <4000 cells/cumm and 33 had total leucocyte count in the range 4001 to 11000 cells/cumm. 3 females had total leucocyte count >11000 cells/cumm. The transgender patient had total leucocyte count in the range 4000 cell to 11000 cells/cumm.

19 patients with the CD4 count in the range 351-500 cells/µl had total leucocyte count in the range 4001 to 11000 cells/cumm. Only 2 patients in this group had a total leucocyte count of more than 11000 cells/cumm. 22 patients with CD4 count >500 cells/µl had total leucocyte count in the range of 4001 cells to 11000 cells/cumm. Pearson chi-square value is 6.205, and the p value is 0.015. So leukopenia has the significant statistical relationship with the low CD4 count. In the study, most

males (43%) and females (36%) had a lymphocyte count in the range of 21%-50%.

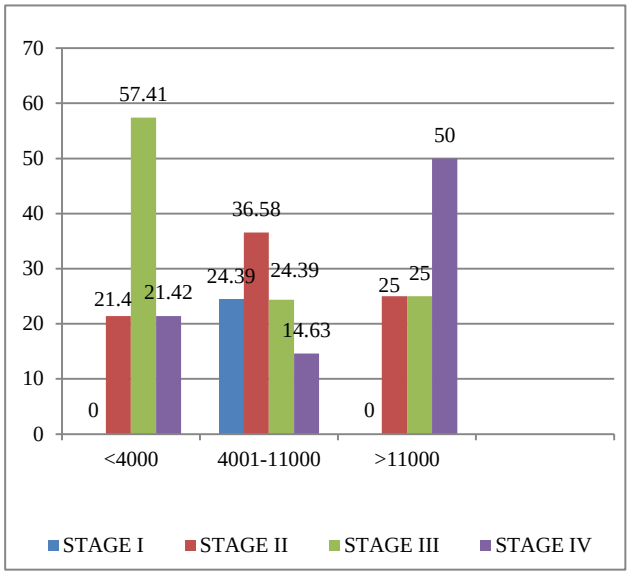

Figure 1: Leukocyte count and WHO staging.

Only 7 patients were found to have neutropenia in the study, of which 3 were males, and 5 were females. 17 patients had the neutrophil count >70% (shift to the left). 75 patients had the neutrophil count in the normal range. Out of the 8 patients with the decreased neutrophil count, the majority (50%) were in stage II. P value is 0.7 ($p>0.05$). The neutrophil count did not have the significant relationship with WHO clinical staging.

Table 4: Neutrophil count distribution.

Neutrophil count	Males	Females	Transgender
<40	3	5	0
41-70	40	34	1
>70	13	4	0

In this study, 23 patients (23%) had platelet count <1.6 lakhs/mm3. Among the patients with thrombocytopenia, the maximum number of patients were in stage IV (47.1%). The Chi-square is 13.552. P value is 0.035 ($p<0.05$). Thus the prevalence of thrombocytopenia increases as the clinical stage advances (Table 5).

Table 5: Platelet count and who staging.

Platelet count lakhs/mm^3	Stage I		Stage II		Stage III		Stage IV	
	No	%	No	%	No	%	No	%
<1.6 (n=23)	1	5	8	23.5	6	20.7	8	47.1
1.61-4.15 (n=72)	16	80	25	73.5	22	75.9	9	52.9
>4.15 (n=5)	3	15	1	2.9	1	3.4	0	0

63 patients had prothrombin time <12.7 seconds. 37 patients had prothrombin time in the normal range (12.7-15.4). In the study, 5 patients had activated plasma thromboplastin time <26.3 seconds. Most patients had APTT (92%) in the normal range. Only 3 patients had prolonged APTT.

Peripheral smear study showed normochromic normocytic in 65% of patients. Dimorphic anemia was seen in 20% of patients. Hypochromic microcytic anemia was seen in 20% of patients.

13% of patients showed eosinophilia. One patient showed a teardrop cell. Transformed lymphocytes were seen in 13% of patients and transformed monocyte in one patient.

Hypersegmented polymorphs were in 30% of patients and rouleaux formation in 5% patients. Target cells were seen in 5% of patients. The shift towards the left was seen in 17% of patients. Reactive lymphocytosis was seen in 5% of patients.

Anisocytosis was seen in 4% and elliptocyte in 3% of patients.

DISCUSSION

Males formed the major group as in a study by Dikshit in PGI Chandigarh. Out of the 100 patients, 78% of patients were 26 yrs to 50 yrs of age consistent with the study by Mgogwe et al in Africa.[7,8] 92% study group were heterosexuals. Patients in WHO clinical stage II formed the major group in the study. 22% patients had the CD4 count above 500 cells/µl as in a study by Parinitha et al in Karnataka.[1] 15 patients (15%) presented with sexually transmitted infection in the study. A study by Turbadkar et al showed that 9.1% of HIV infected patients had syphilis. 64% of patients had various types of dermatoses.[9] Sanjay et al in Maharashtra found out that 90% HIV infected patients develop dermatological manifestation during the disease.[10] The commonest was oral candidiasis seen in 23% of patients. Many patients with candidiasis showed evidence of hematological abnormality of more than one cell lineage. 28% patients had papular pruritic eruptions in a study by Halder et al in West Bengal.[11]

45% patients had the fever at presentation. Fatigue prevalence ranges from 10% to 30% in HIV infected patients.[12] In this study, fatigue was the presenting feature

in 16% of patients. Fatigue is the primary symptom of anemia in HIV infected patients. A study by Cleeland et al showed that maximum improvement in the quality of life occurred when hemoglobin level increased from 11 gm/dl to 12 gm/dl.[12] Anemia was detected in nearly 72% of patients. 67.8% of male patients were anemic, and 79% of female patients were anemic. A study by Sullivan PS et al. showed anemia prevalence between 63% and 95%.[13] Chigs et al showed the prevalence ranged from 70% to 87%.[14] Our results are identical to these studies.[14]

WHO defines anemia as a hemoglobin level <13 gm/dl in males and hemoglobin level <12 gm/dl in females.[15] In this study, patients with CD4 count <200 cells/µl had more severe anemia. 51.4% of patients with CD4 count > 200 cells/µl had normal hemoglobin level.

Patients in WHO clinical stage I (50%) had hemoglobin level in the range 9.1 gm/dl to 13 gm/dl. In advanced stages, none of the patients had a normal hemoglobin level. The chi-square value is 24.012, and the P value is 0.004 ($p<0.05$). This shows that WHO staging and hemoglobin level has the statistically significant correlation. A study by Kasthuri et al showed that the frequency of anemia increases from asymptomatic stage to stage IV.[16]

100% stage IV male patients were anemic. Only 28.6% stage I male patients was anemic. So the level of anemia in males has a significant correlation with WHO staging with P value 0.002.

75% stage I and 88.9% stage IV female patients were anemic. 73% male patients with CD4 count < 350cells/µl had anaemia. Only 58.3% male patients with CD4 count >500 cells/µl had anaemia.

66.6% of patients with CD4 count >500 cells/µl were anemic in a study by Kasthuri et al. 71.4% patients with CD4 count >500 cells/µl showed anemia in our study. Thus there is the correlation between anemia and the immunological status.[16]

14 patients (14%) had leukopenia. A study by Denue et al showed the prevalence of leukopenia in HIV infected patients to be about 5.5%.[17] In a study by Kaloutsi et al, normal leucocyte count was found in 70.4% patients similar to our study.[18] In another study by Patwardhan et al, 75.6% of patients had normal leucocyte count.[19] The degree of leukopenia correlated with disease severity.[20]

85% patients with leukopenia had CD4 count <350 cells/µl. None of the patients with the CD4 count above 500 cells/µl had leukopenia. Thus, leukopenia has the statistically significant correlation with the CD4 count with p value 0.015.

None of the patients in stage I had leukopenia. 10 patients in stage III and stage IV showed leukopenia. The relationship between total leucocyte count and WHO clinical staging is statistically significant (p value 0.047).

16% of patients showed lymphocytopenia. Only 4 patients had lymphocytosis. Majority of the patients (80%) had normal relative lymphocyte count. None of the patients with CD4 count >500 cells/ µl had lymphocytopenia. 50% patients with lymphocytosis had CD4 count >500 cells/µl. The chi-square value is 6.364, and the p value is 0.04 ($p<0.05$) which is significant. 37.5% of patients with lymphocytopenia were in stage IV. Lymphopenia was seen in 25.7% of patients in a study by Choi et al in Seoul.[21] This study also showed more incidence of lymphocytopenia in stage IV patients.[21] Neutropenia was detected in 8% of patients. Neutrophilia was seen in 17% of patients. The prevalence of neutropenia was 24% in a study by Erhabor et al in Nigeria.[22]

The hematological abnormalities are important as they increase the risk of opportunistic infections.[23]

87.5% patients with neutropenia had opportunistic infections. Pancytopenia patients had 100% incidence of opportunistic infection. The majority (47.1%) of patients in thrombocytopenia were in stage IV. 80% of patients with normal platelet count were in stage I. Thrombocytosis was commonly seen among stage I patients. P value is 0.035 ($p<0.05$). Thus there is the statistically significant association between platelet count and the WHO clinical staging. As the stage advances platelet count decreases. 69.5% of patients with thrombocytopenia had CD4 count <350 cells/µl. The prevalence of thrombocytopenia was 18% in a study by Parinitha et al. in India.[1] Patwardhan et al and Costello et al reported a prevalence of 13%.[19] Erhabor et al showed the prevalence to be 12%.[22] The prothrombin time was normal in all patients. APTT was prolonged in 3 patients.

Pancytopenia (anemia, neutropenia, and thrombocytopenia) was identified in 5% of patients. Anemia along with leukopenia was seen in 8% of patients. Neutropenia with thrombocytopenia was seen in 2% of patients. Anemia with thrombocytopenia was seen in 12% of patients.

65% of peripheral smear studies showed normochromic normocytic. In a study by Kasthuri et al, normochromic normocytic was found in 71% of patients.[16] A study by J Mgogwe et al showed 64% normochromic picture similar to our study.[8] This predominant normochromic peripheral smear picture is suggestive of anemia of chronic disease.[24,25] Dimorphic anemia picture was seen in 22% of patients as in a study by Kasthuri et al.[16] The shift towards the left in the granulocytes was seen in 13% of patients. Erbahor et al in Nigeria showed that a shift in the granulocytes is a common finding.[22]

CONCLUSION

HIV infection is a chronic disorder rather than an incurable disease. Factors which determine the outcome of treatment will affect the morbidity. This study has highlighted the higher incidence of hematological abnormalities in HIV infected patients and their

correlation with WHO clinical staging and immunological status. Evaluating and treating hematological abnormalities will greatly decrease the morbidity and further disease progression in HIV infected patients.

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