Study of Bone Marrow Abnormalities in Patients with HIV Disease

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Abstract

Aim : Present work was carried out to study the bone marrow abnormalities in patients with HIV / AIDS and to find their association with peripheral hematological abnormalities.

Methods : Seventy four patients of HIV / AIDS were included in the study. The patients had anemia, leucopenia, thrombocytopenia or pyrexia of unknown origin (PUO) as indications for bone marrow examination. A complete blood count, relevant biochemical investigations, HIV RNA load and CD4 positive lymphocyte counts were done, besides a thorough history and clinical examination. HIV positive patients were classified as those having AIDS and those without AIDS according to NACO criteria.

Results : Majority of patients (72.9%) had AIDS. Bone marrow was normocellular in 78.95% of non-AIDS and 74.55% of AIDS, hypocellular in 5.26% of non-AIDS and 7.27% of AIDS, hypercellular in 15.79% of non-AIDS and 18.18 % of AIDS patients. Myelodysplasia was present in 21.05% of non AIDS and 36.46% of AIDS and the most common series affected was granulocytic (15.79% of total in non-AIDS and 30.9% in AIDS). Dysplasia was statistically significantly associated with lower CD4 count ($p = 0.031$) and anemia ($p = 0.013$). Myelodysplasia was apparent even before patients developed anemia (16.67%). Increased plasma cells in bone marrow were observed in 57.89% of non-AIDS and 65.45% of AIDS, whereas decreased lymphoid cells were seen in 36.84% of non AIDS and 60.00% of AIDS patients.

Conclusions : Myelodysplasia is found in 32.43% of cases of HIV / AIDS and is more common in AIDS than in non AIDS patients. Granulocytic series is most commonly associated with evidence of dysplasia. Myelodysplasia is more common in patients with CD4 count <200 / µl and in patients with anemia. 54.05% of patients had decreased lymphoid cells in bone marrow and it was more commonly seen in AIDS than in non AIDS. ©

INTRODUCTION

Acquired Immunodeficiency Syndrome (AIDS) was first recognized in the United States in 1981 and human immunodeficiency virus (HIV) was isolated from a patient with lymphadenopathy in 1983. By 1984, HIV was clearly demonstrated to be the causative agent of AIDS. The dissemination of HIV-1 in humans represents a catastrophic example of genomic transmission and expansion. Over 60 million individuals have been infected so far leading to nearly 20 million deaths and 20 million orphaned children.[1,2] It is estimated that most of the 16,000 individuals, newly infected each day, live in developing countries.

Bone marrow abnormalities are found at all stages of HIV disease, increasing in frequency as the disease progresses. Infection of marrow mesenchymal stem cells with HIV has been incriminated as an important factor causing bone marrow defects.[3] As a result of HIV infection, the marrow produces a histiocytic reaction which varies from increased number of histiocytes to a full blown hemophagocytic syndrome with severe pancytopenia.[4] Several defects in bone marrow progenitor cells have been described. Reduced colony growth factor has been demonstrated for granulocyte macrophage progenitor cells, multipotential hematopoietic progenitor cells, and megakaryocytic progenitor cells as well as early erythroid progenitor cells in most patients with AIDS.[4]

Marrow from HIV-infected patients is sometimes difficult to aspirate and the trails are of decreased cellularity. The true marrow cellularity is better appreciated on trephine biopsy which is hypercellular in a majority of patients.[5] Although, erythroid dysplasia has been reported to be the most common finding, abnormal granulocytic and megakaryocytic

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development are encountered in approximately one-third of patients.[6] Few studies have shown dysgranulopoiesis to be more frequent and more accentuated than other kinds of dyshematopoiesis.[7] Intense vacuolization, especially in the granulocytic series, is frequent. Dyserythropoiesis may manifest as florid megaloblastic change. This was found unrelated to serum cobalamin and folate levels or to drug therapy with zidovudine or folate antagonists, although these drugs may accentuate it.[8] Granulocytic dysplasia may be apparent at all stages of maturation, with megaloblastic change, nuclear abnormalities and Pelger cells reflecting dysfunctional nuclear maturation.[9] Erythroblasts are often bi - or multinucleated with an irregular nuclear outline and basophilic stippling. Abnormal sideroblasts including ringed forms may be present. Dysplastic features in megakaryocytes are common, occurring in one-third of marrows and include nuclear hypolobulation and micromegakaryocytes.[10,11]

Plasmacytosis has also been reported to occur in HIV infection.[12] Granulomas are an infrequent finding in bone marrow biopsies and may be associated with a broad spectrum of infectious and non-infectious disorders.[13]

We are beginning to learn various clinical manifestations and abnormalities in patients with HIV disease in India. There is a possibility that the manifestations in Indian patients may not be the same as reported from other countries like USA and Africa. Hence, it is imperative to methodically observe and follow clinical and laboratory aberrations in such patients in order to improve our diagnostic and therapeutic skills pertinent to HIV / AIDS. Here, we aimed at studying the bone marrow abnormalities in patients with HIV disease and to investigate their association with peripheral blood abnormalities.

Materials And Methods

The study population included 74 HIV +ve symptomatic or asymptomatic patients. HIV was diagnosed by ELISA method as per NACO guidelines.[14] The study was conducted in Department of Medicine and Department of Pathology, King George Medical University, Lucknow.

Inclusion criteria: HIV+ve patients who had anemia, leucopenia, thrombocytopenia or pyrexia of unknown origin and gave consent were included in the study.

Exclusion criteria: Patients who had chronic liver disease or renal disease other than explained by HIV or primary hematological abnormalities were excluded.

Detailed history was taken which mainly included age, sex, place of residence, occupation, history of blood or blood product transfusions, high risk behavior, fever, weight loss, diarrhoea, oral or genital ulcerations, bleeding diasthesis or history suggestive of systemic involvement. All patients were subjected to thorough physical examination both, systemic and general. Patients were classified into two clinical groups according to NACO criteria.[14]

1. AIDS: (Those patients who fulfilled diagnostic criteria of AIDS according to NACO guidelines)
2. Non AIDS: (Asymptomatic and symptomatic, who did not fulfill the NACO AIDS criteria)

Investigations : Bone marrow examination was performed for indication of anemia, leucopenia, thrombocytopenia or pyrexia of unknown origin. Posterior superior iliac spine was chosen as the site for bone marrow aspiration and biopsy because of large marrow space and least painful site. In obese and old patients, sternum was used for bone marrow aspiration. A smear was drawn and leishmans stained preparation was made. Bone marrow sample was examined for cellularity, morphology including dysplastic changes, fibrosis, granuloma and iron stores. Other investigations performed were hemoglobin, total leucocyte count, differential leucocyte counts, absolute neutrophil, lymphocyte, monocyte, eosinophil and basophil counts, general blood picture, platelet count, reticulocyte count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and total red blood cell count. CD4 and CD8 lymphocyte counts were measured using flow cytometry. HIV RNA load in plasma by RT PCR (SRL Mumbai) wherever possible, were also done. Other relevant investigations where necessary were done in individual patient.

Statistical Analysis : The data were analysed using mean, standard deviation, Pearson's chi-square test, student't' test, Kruskal-Wallis test, two samples Wilcoxon Rank Sum test.

Observations And Results

Out of 74 patients, 19 (27.13%) were grouped as nonAIDS and 55 (72.87%) as AIDS (NACO criteria).[14] Peripheral blood examination and bone marrow examination was done in 61 patients with anemia, 5 with leucopenia , 2 patients with thrombocytopenia and 6 with pyrexia of unknown origin .

The commonest age group of patients was 20-40 years with a range and of 20 to 68 years. Mean age of males was 34.26 ± 15.25 in non-AIDS and 34.69 ± 8.00 in AIDS and of females was 30.33 ± 5.02 in non-AIDS and 32.33 ± 6.99 in AIDS group (Fig. 1, Table 1). Male to female ratio was 4:1. Other epidemiological features are shown in Table 1. Hematological and immunological parameters are shown in Table 2 and 3 and there is no statistically significant difference in hematological parameters in both groups (AIDS and non-AIDS). The commonest type of anemia was normocytic normochromic seen in 88.52% (n=54) of patients, microcytic hypochromic in 6.56 % (n=4) of patients and macrocytic in 4.92 % (n=3) of patients.

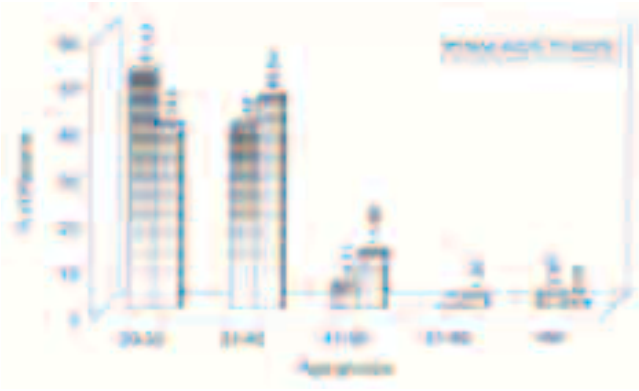

Fig. 1 : Age distribution of AIDS and non-AIDS patients.

Table1 : Epidemiological parameters of patients with HIV/AIDS

Parameter	Total number of patients (N=74) Mean ± SD
Age (male)(n=59)	34.26 ± 15.25(years)
Age (female), (n=15)	30.33 ± 5.02(years)
Commonest mode of transmission in males	Commercial sex worker (78%)
Commonest mode of transmission in females	From husband (96.6%)
Poor socioeconomic status	65.6%

As many as 78.95% of non-AIDS and 74.55% of AIDS patients had normocellular bone marrow and 15.79% of non-AIDS and 18.18% of AIDS patients had hypercellular bone marrow. Bone marrow was hypocellular in 5.26% of non-AIDS and 7.27% of AIDS patients (Table 4).

Myelodysplasia was found in 32.43 % of patients. It was more common in AIDS as compared to nonAIDS patients. As it is evident from Table 5, myelodysplasia was commonest in granulocytic series (27.03%), followed by erythroid (4.05%) and megakaryocytic series (1.35%). Important dysplastic changes observed in granulocytic series were cytoplasmic vacuolations (60%), nuclear dysmorphism (30%), monocytoid cells (1%) and others (1%), in erythroid series were irregular nuclear outline and basophilic stippling (66.67%), megaloblastoid changes (33.33%); in megakaryocytic series were hypolobulation (100%). Out of 24 patients showing dysplasia 20 (83.33%) had anemia, 5 (20.83%) had leucopenia and 1 (4.17%) had thrombocytopenia. Prevalence of dysplasia was more in patients with advanced disease (CD4 count < 200/µl) and anemia (Table 6). Majority of patients (83.33%) with dysplasia had anemia, out of which, only 10% had erythroid dysplasia. Twenty five per cent of patients with granulocytic dysplasia had leucopenia, and all patients with megakaryocytic dysplasia had thrombocytopenia. Nine (34.62%) patients showing dysplasia were on antiretroviral therapy for at least six months.

Increased plasma cells were observed in 57.89% of non-AIDS and 65.45% of AIDS patients, whereas, there was a decrease in lymphoid cells in bone marrow of 6.84% of non-AIDS and 60.00% of AIDS patients (Table 7).

Table 2 : Values and standard deviations of peripheral blood, immunological and bone marrow parameters

Hematological parameters	Non-AIDS Mean ± SD (n=19)	AIDS Mean ± SD (n=55)
Haemoglobin (gm/dl)	11.13± 1.53	10.46± 1.94
Packed cell volume (PCV) (ml %)	21.91± 16.02	27.36 ± 12.40
Total Red Blood Cell Count (TRBC) Million/mm^3	4.41 ± 0.36	3.09 ± 0.36
Mean corpuscular volume (MCV)(fl)	80.47 ± 5.62	81.81± 5.61
Mean corpuscular Hemoglobin (MCH)(pg)	27.36 ± 2.71	27.59 ± 1.81
Reticulocyte count (%)	0.83 ± 0.24	0.96 ± 0.22
Erythrocyte sedimentation Rate (ESR)(mm/hr)	20.06 ± 6.60	18.07± 4.24
Total leucocyte count (TLC) (Cells/mm^3)	7592.06 ± 1523.54	7457.23 ± 1998.02
Lymphocyte count (%)	26.37 ± 11.54	24.34 ± 10.29
Neutrophil (%)	66.57 ±12.69	69.75 ± 11.83
Eosinophil (%)	4.66 ± 7.27	2.96 ± 3.43
Monocyte (%)	1.77 ± 2.25	2.04 ± 2.35
Basophil (%)	0.6 ± 0.2	0.5 ± 0.12
Absolute Neutrophil count x 10^9/L	5.09 ± 1.47	5.04 ± 1.71
Absolute lymphocyte count x 10^9/L	2.08 ± 9.29	1.78 ± 8.36
Absolute eosinophil count x 10^9/L	3.85 ± 5.69	2.20 ± 2.67
Platelets (Lacs/mm^3)	1.91 ± 0.63	1.92 ± 0.49
CD4 count / µl	265.24 ± 97.65	177.53 ± 111.43
HIV RNA in plasma (copies/ ml)	25514.84 ± 14080.04	59566.25 ± 29092.82
Plasma cells (%) in bone marrow	3.89 ± 2.21	5.03 ± 2.82
Lymphoid cells (%) in bone marrow	14.53 ± 8.39	11.14 ± 11.06

Table 3 : Peripheral blood findings in AIDS/HIV

Parameter	Non-AIDS (N=19)	AIDS (N=55)	P Value
Anemia	11(57.89%)	50(90.91%)	0.05
Leucopenia	1 (5.26%)	4 (7.27%)	0.417
Thrombocytopenia	0	2(3.64%)	0.11
Lymphopenia	4(21.1%)	15(27.27%)	0.05

Table 4 : Bone marrow cellularity in HIV/AIDS patients

Bone marrow cellularity	Non-AIDS		AIDS		Total	
	n	%	n	%	n	%
Normocellular	15	78.95	41	74.55	56	75.68
Hypocellular	1	5.26	4	7.27	5	6.75
Hypercellular	3	15.79	10	18.18	13	17.57
Total	19	100	55	100	74	100

Table 5 : Myelodysplasia in bone marrow in HIV patients

Dysplasia	Non-AIDS n (%)	AIDS n (%)	Total n (%)
No dysplasia	15 (78.95)	35 (63.64)	50 (67.57)
Granulocytic	3 (15.79)	17 (30.90)	20 (27.03)
Erythroid	1 (5.26)	2 (2.64)	3 (4.05)
Megakaryocytic	0 (0.00)	1 (1.82)	1 (1.35)
Total	19 (100)	55 (100)	74 (100)

Discussion

Clinical presentation in patients with HIV / AIDS is diverse and it may arise from involvement of almost any body system. Hematological abnormalities are not uncommon in HIV / AIDS. Large percentage of patients with HIV has anemia, leucopenia and thrombocytopenia during the course of disease. According to Paradela A *et al*,[15] 90 % of patients are reported to have bone marrow abnormalities during the course of disease in the form of increased cellularity, dysplasia or granulomatous involvement. 'invitro' studies have shown that HIV can directly infect the hematopoietic and mesenchymal cells and can influence their activity.[3] The exact mechanism of HIV induced bone marrow and peripheral blood changes are not known, however, these are possibly due to either direct effect of HIV, nutritional deficiencies, opportunistic infections or bone marrow suppression by antiretroviral therapy, and other drugs used in the treatment of HIV infection.[8]

Bone marrow aspiration and biopsy is generally needed in patients presenting with hematological abnormalities where the cause could not be discerned. The present study involved 74 patients of HIV / AIDS who had anemia, leucopenia, thrombocytopenia or pyrexia of unknown origin. Clinicoepidemiological features are presented in Table 1 and are similar to the data reported from India and other countries.[16,17] Three fourths (72.97%) of our patients had clinical AIDS while the rest (27.03%) were either asymptomatic or symptomatic but did not fit into criteria of AIDS (Table 1). This data is biased towards symptomatic HIV disease as most patients come to hospital when they become symptomatic.

Eighty two percent of patients had anemia and its prevalence was significantly higher in patients with AIDS than in non AIDS (p=0.05) (Tables 2, 3). The most common type of anemia was normocytic normochromic, which is in agreement with the reports in the literature.[18] Macrocytic anemia is commonly reported in patients on ART.[8] Five percent (3 Patients) in our study had

Table 7 : Variations in lymphoid and plasma cells in bone marrow of HIV patients

	Non-AIDS (n=19) n (%)	AIDS (n=55) n (%)	Total (n=74) n (%)	p value
Lymphoid cells				
Normal	10 (52.63)	19 (34.55)	29 (39.19)	$\chi^2(2)= 3.11$,
Decreased	7 (36.84)	33 (60.00)	40 (54.05)	p = 0.210
Increased	2 (10.53)	3 (5.45)	5 (6.76)	
Plasma cells				
Normal	8 (42.11)	19 (34.55)	27 (36.49)	$\chi^2(1)= 0.35$,
Increased	11 (57.89)	36 (65.45)	47 (63.51)	p = 0.555

Table 6 : Myelodysplasia and its association with peripheral blood abnormalities

Parameters	Granulocytic	Erythroid	Megakaryocytic	Significance
Haemoglobin				
Normal (n=13)	2(15.38%)	1(7.69%)	1 (7.69%)	p=0.01
Decreased (n=61)	18(29.51%)	2(3.28%)	0 (0.0%)	
PCV				
Normal (n=7)	1(14.29%)	1(14.29%)	0(0.00%)	p= 0.74
Decreased (n=67)	19(28.36%)	2(2.99%)	1(1.49%)	
MCV				
Normocytic (n=50)	5 (30%)	2 (4%)	1 (2%)	p= 0.00
Microcytic (n=23)	5 (21.74%)	0 (0.00%)	0 (0.00%)	
Macrocytic (n=1)	0 (0.00%)	1 (100%)	0 (0.00%)	
CD4 count				
<200 (n=31)	10 (56.56%)	1 (33.33%)	0 (0.00)	p= 0.03
200-350 (n=30)	6 (33.33%)	0 (0.00%)	1 (100.00%)	
>350-500 (n=7)	2 (11.11%)	2 (66.67%)	0 (0.00%)	
Total leucocyte count				
Normal(n=69)	15(21.74%)	3(4.35%)	1(1.45%)	p=0.02
Decreased(n=5)	5(100%)	0(0.00%)	0(0.00%)	
Platelets				
Normal(n=72)	20(27.78%)	3(4.17%)	0(0.00%)	p=0.53
Decreased(n=2)	0	0	1(50%)	
HIV RNA in plasma				
<30,000 (n=10)	3(30%)	1(10%)	1(10%)	p= 0.54
30,000-99,000(n=49)	6(32.65%)	1(2.04%)	0 (00.00%)	
>99,000 (n=15)	1 (6.66%)	0 (00.0%)	0 (0.00%)	

macrocytic anemia and all were on ART. The early diagnosis and effective management of anemia in patients with HIV disease is of tremendous importance as anemia in such patients may be an indicator of poor prognosis and progression to advanced disease.[6] Leucopenia was observed in 7.27 % of AIDS and 5.26 % of non AIDS (Table 3) and all patients showed granulocytic dysplasia, emphasizing that the latter was a major cause for leucopenia. Lymphopenia was seen in 21.1% of nonAIDS and 27.27% of AIDS patients. Prevalence of thrombocytopenia in patients with HIV / AIDS has been variably reported in 13 % to 61% cases.[17,18] Our study revealed a prevalence of 3.64% and it was significantly different in AIDS and non AIDS group (Table 3).

Bone marrow was particularly examined for cellularity, fibrosis, dysplasia and for the evidence of granuloma. Previous reports indicate that most of the cases with HIV have hypercellular bone marrow.[15] However present study revealed bone marrow to be normocellular in 75.68%, hypocellular in 6.75% and hypercellular in 17.57% patients (Table 4). The difference is difficult to explain but it is likely to be due to different cohort of patients included in various studies. Majority (72.9%) of patients under study had full blown AIDS where bone marrow could likely be normocellular or hypocellular rather than hypercellular which is predominant in early stages of disease.[20] We have not observed increased reticulin in any of bone marrows examined. This is in accordance with reports by Treacy *et al.* [6] Myelodysplastic changes due to HIV can explain normocytic or macrocytic anemia which is common in patients with AIDS. Myelodysplastic changes in bone marrow were found in 32.43% cases as compared to 50-90 % reported in literature.[15] This difference in incidence of dysplasia in different population of patients is interesting to note. The underlying reason is not clear and this needs to be addressed in future studies involving larger number of patient. Despite the fact that myelodysplasia in majority of patients is directly proportionate to HIV RNA load, the incidence of myelodysplasia is less in the study.[20] Higher incidence of dysplasia in advanced disease is probably due to increased HIV RNA load, cytokine mediated effect of disease and drug related changes and also effect of infections. Myelodysplasia was significantly more common in AIDS (36.36%) than in non-AIDS (21.05%), ($p = 0.01$). Cells most commonly showing dysplasia in our study were granulocytes (27.03%) followed by erythroid in 4.05% and megakaryocytes in 1.35% (Table 5). Some workers have previously reported erythroid dysplasia to be the commonest type of dysplasia whereas others reported it in granulocytic series of cells.[15] Dysplastic changes observed in granulocytic series were cytoplasmic vacuolations (60%), nuclear dysmorphism (30%), monocytoid cells (1%) and others (1%); in erythroid series were irregular nuclear outline and basophilic stippling (66.67%), megaloblastoid changes (33.33%) and in megakaryocytic series were hypolobulation (100%). Most common cause of myelodysplasia could possibly be due to an inherent defect of hematopoietic cells. However some patients are diagnosed to be having secondary myelodysplastic syndrome and important causes include drugs, alcohol, infections like tuberculosis and malaria. Reports are lacking on the incidence of HIV disease in patients of myelodysplastic syndrome. Nevertheless HIV infection should be included in differential diagnosis of patients with secondary myelodysplasia. The myelodysplasia in early stages may not reflect changes in peripheral blood smear and thus may remain undiagnosed. This also suggests that prevalence of myelodysplasia in HIV may be much higher than what is reported. This is evident from our observation that 7.69% of patients showing erythroid dysplasia did not have anaemia. Dysplasia seems to be an important factor in HIV induced leucopenia while it may not be important for causing anemia and thrombocytopenia that may occur due to other factors. No such study is available which has reported the incidence of myelodysplasia based on bone marrow examination in all HIV / AIDS patients irrespective of their hematological presentation.

The cause of fever was determined on bone marrow examination in 2 out of 6 patients. One patient had histoplasmosis and the other had evidence of tubercular bacilli in bone marrow. Brook MG *et al*[21] reported that 27% of marrows in HIV patients with fever revealed the cause, commonest being mycobacteriosis in 20%.

Various studies have reported that plasma cells in bone marrow are increased in all patients with HIV infection.[12] We observed increased number of plasma cells in the marrow of 57.89% of nonAIDS and 65.45% of AIDS patients (Table 7). Mean percent of plasma cells in bone marrow sample of nonAIDS was 3.89 ± 2.21 and 5.03 ± 2.82 in AIDS (Table 2), however, this difference was not statistically significant. Plasmacytosis was not only confined to those with advanced disease in whom opportunistic infections could be implied with but was also seen in patients at an early stage who had no concurrent infection. Increased plasma cells could thus be a polyclonal B cell response to HIV infection and can occur at any stage of HIV disease. Lymphoid cells were found to be decreased in the marrow in 36.84% of non AIDS and 60% of AIDS patients. Mean count of lymphoid cells was 14.53 ± 8.39 percent in nonAIDS and 11.14 ± 11.06 percent in AIDS patients (Table 2). However, it is interesting to note that decreased lymphoid cells in bone marrow was more common in AIDS patients compared to non-AIDS. We presume that decrease in marrow lymphoid cells could possibly explain lymphopenia in HIV patients. Further studies are needed to define the diagnostic and prognostic significance of decreased lymphoid cells in bone marrow of non-AIDS and AIDS

patients. However, we hypothesize that decrease in marrow lymphoid cell pool is consequent upon the destruction of lymphoid cells by HIV.

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