

Bone marrow study in patients with Human Immune Deficiency Virus and Acquired Immune Deficiency Syndrome

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ABSTRACT

Introduction: Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome (HIV/AIDS) can involve almost any organ system. The study was aimed to assess the various bone marrow abnormalities seen in HIV/AIDS patients with haematologic abnormalities. **Materials and Methods:** 43 HIV infected patients were included in the study. Baseline haematological investigations included full blood count, CD4 positive lymphocyte counts, and absolute lymphocyte count. Bone marrow aspiration and trephine biopsy were done in all patients. Bone marrows of these patients were carefully evaluated for any abnormalities. HIV positive patients were classified into AIDS group (76%) and non-AIDS (24%) group using National AIDS Control Organisation (NACO) criteria. **Results:** Normocytic normochromic anaemia was the most common peripheral haematological finding occurring in 72% of patients. The AIDS group had statistically significant lower platelet counts ($p=0.004$) but no differences in the other parameters. Bone marrow was normocellular in 63.6% in the AIDS group and 100% in the non-AIDS group. Dysplasia was observed in 37.2% of patients, predominantly affecting granulocytic series. Myelodysplasia was statistically associated with a low platelet count. Reduced marrow lymphoid precursors (CD4+) were seen in 37.2% of patients. **Conclusions:** Bone marrow abnormalities were common in HIV/AIDS patients with haematological abnormalities. The AIDS group had a statistically significant lower platelet count. Myelodysplasia was found in 37.2% of patients with HIV disease and was also statistically associated with a lower CD4+ lymphocyte count.

Keywords: AIDS related complex, bone marrow diseases, bone marrow examination, HIV infections

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INTRODUCTION

Human Immunodeficiency Virus (HIV) infection can influence all the haematopoietic cell lineages, resulting in a broad spectrum of

haematological abnormalities. Direct invasion of the haematopoietic cells by HIV together with infectious, inflammatory and neoplastic processes explain the various haematological alterations, which are seen during the course of infection.

The consequences of these haematologic problems are twofold. First, they are associated with morbidity in themselves that can adversely alter the patient's quality of life such as from anaemia (fatigue and dyspnoea), leucopaenia (infections) and thrombocytopenia (bleeding). Second, they hinder treatment of the primary viral infection, secondary infections and neoplastic complications. The poor haematopoietic tolerance of therapies often necessitates dose reductions, alteration of drug regimens or interruption of therapies. There are no clear guidelines about the optimal management of these haematologic disorders. Use of marrow stimulants such as granulocyte-colony stimulating factor (G-CSF) and erythropoietin in addition to Highly Active Anti-Retroviral Therapy (HAART) have been shown to have a role in reducing infective complications.^{1, 2}

The study aimed to assess the various bone marrow abnormalities seen in HIV/AIDS patients with haematological abnormalities.

MATERIALS AND METHODS

The study was approved by the local ethics committee and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. All persons gave their informed consent prior to their inclusion in the study.

An observational study was carried

out on a cohort of patients aged 16 years and above (either sex) presenting to our hospital who were Enzyme-linked immunosorbent assay (ELISA) positive for HIV and who had peripheral haematological abnormalities like anaemia, leucopaenia, neutropaenia, lymphopenia and thrombocytopenia over a period of three years. A total of 43 patients were studied. Detailed clinical history was obtained and physical examination was carried out.

The following haematological investigations were carried out for all patients: haemoglobin, total leucocyte count (TLC), differential leucocyte count (DLC), erythrocyte sedimentation rate (ESR), platelet count, mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), packed cell volume (PCV), reticulocyte count, peripheral smear for blood picture, bleeding time, clotting time, and serum urea, serum creatinine. Absolute lymphocyte count was calculated as $ALC (/mL) = TLC \times DLC (\%)$.

Diagnosis of HIV was made in these patients by ELISA and Western Blot (2 ELISA using different kits or 1 ELISA and 1 Western Blot). CD4 count was carried out on all patients. The bone marrow aspirate was used to assess the cellularity. Bone marrow aspiration and biopsy: aspirate was stained using Leishman's stain, whereas trephine biopsy was stained with haematoxylin and eosin stain. Ziehl-Neelsen (ZN) staining for Acid Fast Bacillus (AFB) was done in selected cases. All the bone marrow aspiration and biopsy specimens were examined and interpreted by the same pathologist, to avoid inter-observer variation.

Patients were classified into two groups – AIDS and non-AIDS according to National AIDS Control Organisation (NACO) criteria and all the patients were categorised based on the Center for Disease Control (CDC) and Prevention clinical category of HIV Infection as shown in graph (Figure 1).^{3, 4} Patients who were on antiretroviral therapy were excluded. This was done to reduce the confounding effect of antiretroviral drug induced bone marrow suppression.

Statistical Analysis: Proportions were compared using chi-square test of significance. Student t test was done as indicator of statistical significance. Data analysis was carried out using Statistical Package for Social Science (SPSS Version 10.5, Chicago, IL, USA.)

RESULTS

The demographic data of the patients are shown in Table 1.

The distribution of mean values of laboratory parameters among study groups is shown in Table 2.

In five cases, adequate bone marrow could not be aspirated, in two cases it was grossly diluted with blood. In such patients, the trephine biopsy was relied upon for cellularity. There was no statistically significant difference in cellularity between the two groups ($p=0.08$). The results of marrow cellularity are depicted in Table 3. Overall, the bone marrow biopsy was normal in all non- AIDS group but only in 27 (81.8%) AIDS group. Figure 2 shows a hypocellular bone marrow.

In both groups, the majority of patients had normal lymphoid precursors. In the AIDS group decreased lymphoid cells were seen in 13 (39.4%) patients and was increased in two (6.1%) patients. In the non-AIDS group decreased lymphoid cells were seen in three (30%) patients whereas these were increased in one (10%) patient. There was no statistically significant difference in the number of lymphoid cells between the two groups ($p=0.821$).

Increased plasma cells were observed in 48.5% patients in the AIDS group and 60%

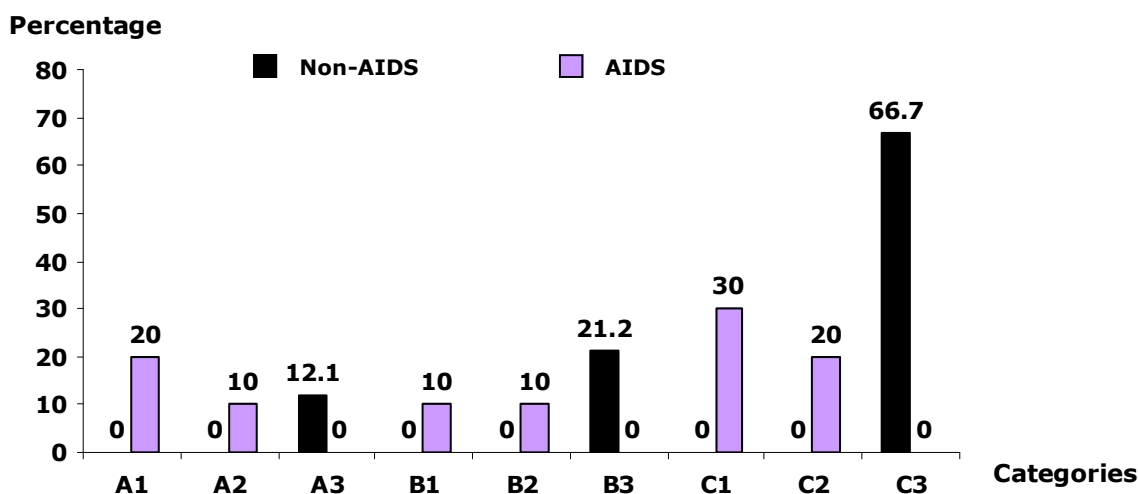


Fig. 1: Distribution of patients based on the Center for Disease Control (CDC) and Prevention classification for HIV infection (Refer to supplementary text for Classification).

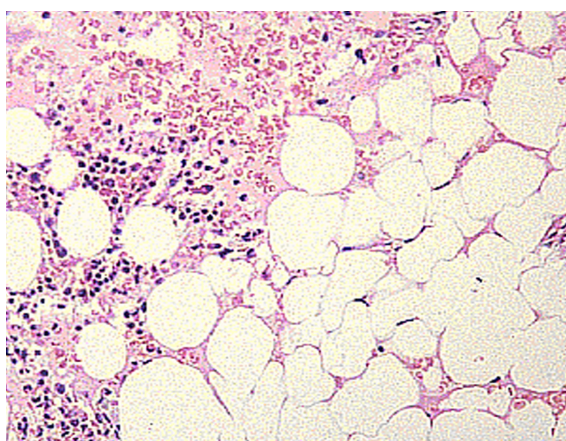


Fig. 2: Bone marrow biopsy showing a hypocellular marrow.

patients in the non-AIDS group.

In the AIDS group, myelodysplasia was seen in 14 (42.4%) patients. It was commonest in the granulocytic series (27.3%), followed by the erythroid (9.1%) and megakaryocytic series (3%). Trilineage dysplasia was noted in one patient. In the non-AIDS group, myelodysplasia was seen in two (20%) patients involving the granulocytic series. There was no statistically significant difference in myelodysplasia between the two groups ($p=0.683$). Bone marrow aspirate in

figure 3 shows myelodysplasia with ring shaped nucleus in a neutrophil.

Although myelodysplasia was more common in patients with lower CD4 lymphocyte counts (<200 cells/mL), it did not show any significant association ($p=0.683$) (Table 3). Myelodysplasia showed no significant association with total leukocyte count ($p=0.550$). However it showed significant association with thrombocytopenia ($p=0.007$) (Table 4).

Among the other abnormalities seen in the AIDS group, granulomas suggesting miliary tuberculosis were seen in three (9.1%) patients, focal fibrosis was seen in one patient and hypoplastic bone marrow with fibrosis was seen in two patients. The three patients with granulomas also had clinical features of miliary tuberculosis.

DISCUSSION

Haematological abnormalities and morphological changes in the bone marrow of patients with HIV infections have been reported

Table 1: Demographic profile of the study population.

Parameters	Overall (n=43)	Non-AIDS (n=10)	AIDS (n=33)	p value
Mean age (yrs)	37 ± 7.9	37 ± 7.9	37.9 ± 7.6	0.219
Age groups				
20-29	9 (20.9)	4 (40)	5 (15.2)	0.269
30-39	18 (41.9)	3 (30)	15 (45.4)	for trend
40-49	12 (27.9)	3 (30)	9 (27.3)	
>50	4 (9.3)	0 (0)	4 (12.1)	
Gender				
Male	34 (79.1)	6 (60)	28 (84.9)	0.091
Female	9 (29.9)	4 (40)	5 (15.1)	
Mode of HIV transmission				
Multiple sexual partners	24 (55.8)	5 (50)	19 (57.6)	0.945
Infected partner	3 (7.0)	1 (10)	2 (6.1)	for trend
Blood products	5 (11.6)	1 (10)	4 (12.1)	
Unknown	11 (25.6)	3 (30)	8 (24.2)	

Table 2: Distribution of mean values of laboratory parameters among study groups.

Haematological parameters	Overall (n=43)	Non-AIDS (n=10)	AIDS (n=33)	p value
Haemoglobin (gm/dL)	9.8 ± 1.63	10.4 ± 1.3	9.6 ± 1.7	0.122
Neutrophil (%)	68.8 ± 9.7	67.6 ± 8.5	69.2 ± 10.1	0.621
Lymphocytes (%)	27.8 ± 8.8	28.7 ± 8.1	27.6 ± 9.1	0.722
Eosinophil (%)	2.0 ± 1.25	1.8 ± 0.9	2.1 ± 1.3	0.492
Monocytes (%)	1.7 ± 1.3	1.6 ± 1.2	1.8 ± 1.4	0.675
Basophils (%)	0.1 ± 0.3	0.2 ± 0.4	0.3 ± 0.4	0.122
Reticulocytes (%)	0.9 ± 1.1	0.9 ± 0.2	0.8 ± 0.3	0.329
Platelets (10 ⁹ /L)	1.84 ± 0.47	2.20 ± 0.32	1.77 ± 0.45	0.004

in many studies. These abnormalities are seen not only in patients with advanced disease, but also during primary infection and during the clinical latency phase.⁵ They progress in frequency and severity from asymptomatic carrier state to AIDS-Related Complex (ARC) to symptomatic AIDS. Low blood counts are common in patients with HIV infection and AIDS, with anaemia, leucopenia and thrombocytopenia each developing in more than half of infected patients.⁶ Isolated thrombocytopenia that resembles idiopathic thrombocytopenic purpura (ITP) sometimes occurs early in the illness. Cytopenias often cause symptoms and contribute to the complications suffered by patients with AIDS like infections, anaemia and bleeding. These

cytopenias are often treatable and even correctable.

In our study, 43 HIV positive patients were studied of which 33 had AIDS. Inadequate aspiration of the bone marrow was seen in 11% cases. It may be a consequence of focal fibrosis, which was seen in 14% of cases. This finding is in agreement with the report of Tripathi *et al.* and Sitalakshmi *et al.* who observed marrow fibrosis in 54% HIV infected cases, whereas Karcher *et al.* have documented marrow fibrosis in 20% cases.⁷⁻⁹

Various peripheral haematological abnormalities were observed in isolation or in combination. Normocytic anaemia was the most common haematological abnormality, occurring in 76% patients (54.5% AIDS patients and 60% of non-AIDS patients) and this is in agreement with the published literature.^{7, 8} Normocytic anaemia probably reflected anaemia of chronic disorders like recurrent pneumonia, tuberculosis and opportunistic infections involving the bone marrow. Myelodysplastic changes in the bone marrow can also cause normocytic anaemia.^{10, 11} Microcytic anaemia was observed in 20% of the patients in our study (21% the AIDS patients and 20% of the non-AIDS patients). It was

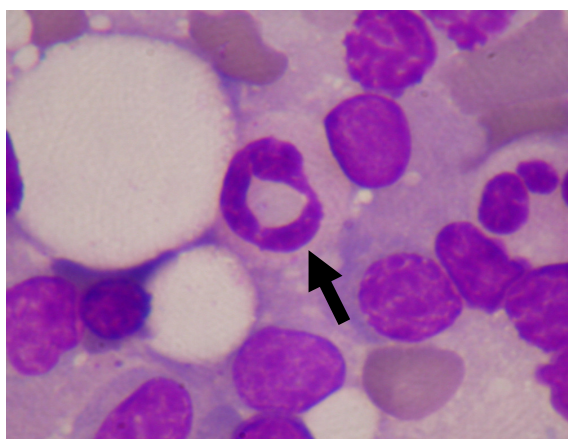


Fig. 3: Bone marrow aspirate showing myelodysplasia with ring shaped nucleus in a neutrophil (Arrow).

Percentages

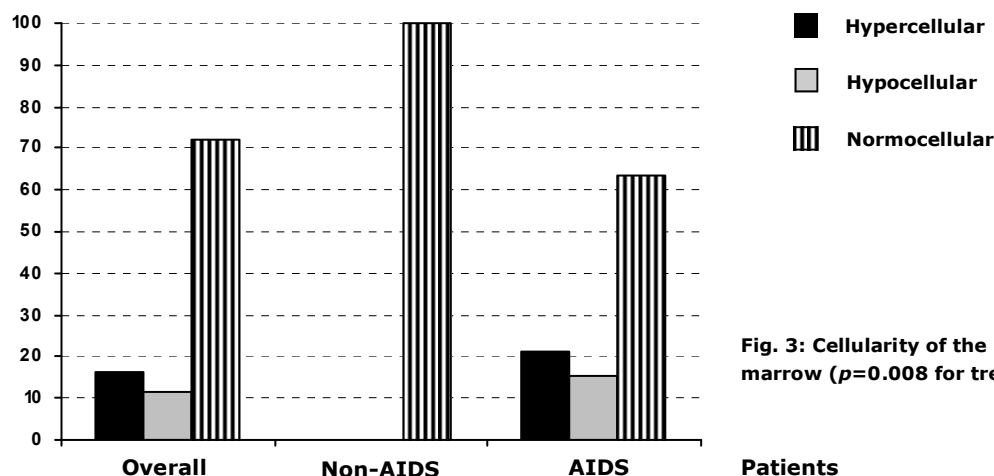


Fig. 3: Cellularity of the bone marrow ($p=0.008$ for trend).

more common in women. Lymphopenia was seen in 12% of patients belonging to the AIDS group as compared to 27% AIDS patients in another study.⁷ Lymphopenia is probably a result of direct attack of lymphocytes by HIV through CD4 binding sites.¹² We observed leukopenia in 14% of patients (12% patients of AIDS group and 20% patients in non-AIDS group). Tripathi *et al.* reported leukopenia in 6% of patients (7% patients in AIDS group and 5% patients in the non-AIDS group).⁷ This difference in percentage can be attributed to the difference in the sample size of the study groups. Leukocytosis was seen in six percent of patients with AIDS. These patients had sepsis secondary to pneumonia or urinary tract infection.

Pancytopenia was observed in three percent of patients in the AIDS group. Studies have reported pancytopenia in 23% of HIV positive patients.⁸ ITP was observed in three percent of patients in the AIDS group which is comparable with other studies.^{7, 8} We also observed a statistically significant lower platelet count in the AIDS group compared to the non-AIDS group ($p<0.05$).

We studied bone marrow for cellularity, dysplasia, plasma cell numbers, lymphoid precursor numbers, dysplastic changes, fibrosis and granulomas. The majority of HIV infected patients (63% in the AIDS group and 100% in the non-AIDS group) had a normocellular bone marrow. However mixed reports

Table 3: Correlation between myelodysplasia and CD4 lymphocyte count.

	CD4 lymphocyte count	Myelodysplasia					Total
		Erythroid	Granulocytic	Megakaryocytic	Nil	Trilineage	
	<200	3 (9.1)	9 (27.3)	1 (3.0)	19 (57.6)	1 (3.0)	33 (100)
	≥200	0 (0)	2 (20.0)	0 (0)	8 (80.0)	0 (0)	10 (100)
Total		3 (7.0)	11 (25.6)	1 (2.3)	27 (62.8)	1 (2.3)	43 (100)

are available on this finding, as some studies concur while others disagree with our data.^{7, 9, 13, 14} A hypercellular marrow may be seen in early stages of the disease, but it is more likely to be normocellular or hypocellular in advanced disease.¹⁵

Myelodysplasia was also one of the parameters assessed in the bone marrow aspirates of our study population as various studies have reported it to be a common feature in AIDS patients.⁹ Myelodysplasia was observed in 37% patients (42.4% patients in the AIDS group and 20% patients in the non-AIDS group). Studies have reported myelodysplasia in 32.3% patients (37% patients in the AIDS group and 21% patients in the non-AIDS group), which had a significant association with a low CD4 lymphocyte count.⁷ However Karcher *et al.* reported myelodysplasia in 69% of HIV positive patients.⁹ In our study, dysplastic changes predominantly involved the granulocytic series followed by the erythroid and megakaryocytic series. Trilineage dysplasia was seen in 3% cases, all of which belonged to the AIDS group. Our findings agreed with another study observation.⁷ Although we found clustering of myelodysplasia cases with low CD4 lymphocyte counts, we could not establish any statistical association.

We also observed increased plasma

cells in the bone marrow in 27% of HIV infected patients (49% patients of AIDS group and 60% patients of non-AIDS group). Other studies have reported plasmacytosis in 25% and 22% patients respectively.^{9, 16}

Reduced bone marrow lymphoid cells were seen in 37% HIV infected patients (39% patients of AIDS group and 30% patients of non-AIDS group). These findings were similar to another study.¹⁰

Interestingly, granulomas were seen in 9% of patients in the AIDS group who also had clinical features suggestive military tuberculosis. In all these cases, bone marrow stained negatively for acid-fast bacilli. Castella *et al.* and Calore *et al.* reported granulomas in 16% and 12% cases respectively.^{14, 17}

Treatment of haematological abnormalities aims primarily at reducing replication of HIV, thereby diminishing suppression of haematopoiesis by the virus, and at controlling the opportunistic infections during the course of the disease. The possibility of bone marrow suppression mediated by a toxic drug effect should be considered in these patients. In our study, we only studied patients who had not been previously treated with any HIV therapy. These haematologic complications of HIV infection will undoubtedly decrease as infectious complications are better controlled,

Table 4: Correlation between myelodysplasia and platelets count.

		Erythroid	Granulocytic	Myelodysplasia Megakaryocytic	Nil	Trilineage	Total
Platelet count	Low	3 (9.1)	9 (27.3)	1 (3.0)	19 (57.6)	1 (3.0)	33 (100)
	Normal	0 (0)	2 (20.0)	0 (0)	8 (80.0)	0 (0)	10 (100)
Total		3 (7.0)	11 (25.6)	1 (2.3)	27 (62.8)	1 (2.3)	43 (100)

resulting in longer life spans.

The finding of cytopenias in conjunction with a hypercellular marrow with dyspoietic features which are described in myelodysplasia, increased megakaryocytes forming clusters and fibrosis which are consistent with myeloproliferative disease, increased plasma cells, eosinophils, increased iron and fibrosis which are suggestive of chronic infection are all encountered in HIV infection and hence pose diagnostic confusion. With the continuing rise in prevalence of HIV infection worldwide, it is important for the haematopathologist to recognise the haematological abnormalities and morphological changes in the bone marrow associated with HIV infection.

The exact mechanisms of HIV induced peripheral haematologic and bone marrow abnormalities are not known. The aetiology of these findings are possibly either direct effects of HIV, nutritional deficiencies, opportunistic infections of marrow or the use of marrow suppressive agents. Further research on haematological complications of HIV disease will lead to effective management of cases and reduce the morbidity and mortality from this dreaded disease

There are several limitations with our study. First the sample size was small. More over we did not culture the bone marrow aspirate for detecting opportunistic pathogens and HIV RNA estimation was not done in our patients.

In conclusion, peripheral and bone marrow abnormalities are common in HIV infected individuals and patients with AIDS.

These abnormalities become more frequent as the disease progresses. Bone marrow study is an important investigation in HIV infected patients with peripheral haematological abnormalities. It is a relatively safe and low cost procedure that can contribute to a comprehensive evaluation of cytopenias which lead to various complications.

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