

Research Article

Morphological study of bone marrow in HIV/AIDS patients with anaemia

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Abstract

Background and Objectives: Human immunodeficiency virus can involve almost any organ system. Bone marrow changes include varying degree of dysplasia in one or more cell lines, plasmacytosis and granuloma. Anaemia has been found to be the most common hematological manifestation in HIV/AIDS patients in many studies. There is hardly any study that describes the bone marrow changes in HIV infected anaemic patients. Hence, present study was aimed to study the bone marrow morphology in HIV infected anaemic patients and to evaluate iron stores.

Materials and Methods: Seventy five HIV positive patients with haemoglobin ≤ 10 gm/dl and aged between 18-58 years were enrolled in the study. The study was conducted from November 2011 to September 2013. A detailed clinical history and examination was done. Complete hemogram and CD4+ counts were done in all patients and were classified as those having AIDS and Non AIDS, according to NACO criteria. Written informed consent was taken from patients and bone marrow aspiration was done from posterior superior iliac spine. Smears were stained with Leishman stain for morphological study and Perl's Prussian blue for iron stores.

Results: Total patients included in the study were 75. Majority of patients had normocytic normochromic anaemia seen in 58.66%. Normocellular bone marrow was most common seen in 63.66% patients. Dysplasia was seen in 76% patients. Erythroid dysplasia was the most common dysplasia seen in 68%. Granuloma was seen in one case. Iron stores were found to be adequate in majority of patients.

Conclusion: Bone marrow changes were common in HIV/AIDS anaemic patients. Normocytic normochromic anaemia was the most common peripheral smear finding. Hypocellular bone marrow was more common than hypercellular marrow in advanced stage of disease. Dysplastic changes were more common in AIDS than Non AIDS. Erythroid dysplasia was the most common type of dysplasia. Iron stores were adequate in most of the cases as in anaemia of chronic disease.

Keywords: Anaemia, Bone marrow, HIV

1. Introduction

Human immunodeficiency virus infection has emerged as a major health problem worldwide. Acquired immunodeficiency virus was isolated from a patient with lymphadenopathy in 1983. By 1984, HIV was clearly demonstrated to be the causative agent of AIDS¹. India has an estimated 2.5 million HIV infection and worldwide approximately 2.7 million people are getting newly infected with this virus every year². Hematological abnormalities are common in patients with HIV/AIDS. The common findings in peripheral blood include thrombocytopenia, leucopenia, anaemia or pancytopenia. Bone marrow findings include trilineage dysplasia, increased eosinophils and plasma cells, increased iron and reticulin fibrosis. These abnormalities may be due to direct toxic effect of virus on progenitor cells, ineffective hematopoiesis, immune mechanism and drug reactions³.

Infection of marrow mesenchymal stem cells with HIV has been incriminated as an important factor causing bone marrow defects⁴. As a result of HIV infection, the marrow produces a histiocytic reaction which varies from increased number of histiocytes to full blown hemophagocytic syndrome with severe pancytopenia⁵. Several defects on bone marrow progenitor cells have been described. Reduced colony growth factor has been demonstrated for granulocyte macrophage progenitor cells and megakaryocyte progenitor cells in most patients with AIDS¹.

The present study was done to study the morphology of bone marrow in HIV/AIDS patients with anaemia and to assess iron stores.

2. Materials and Methods

A prospective descriptive clinical study of 75 HIV/AIDS patients with clinically evident anaemia or haemoglobin ≤ 10 gm/dl, aged 18-58 years, reporting to antiretroviral therapy centre and admitted to a tertiary care institution of India were taken for the study, during a period between November 2011 to September 2013. Pregnant and severely thrombocytopenic patients with platelet count of $\leq 15,000/\mu\text{L}$ were excluded from the study. The study got clearance from institutional ethical committee.

HIV confirmation was done by three tests- SD-Bioline, Comb-AIDS and Retrochek. CD-4 count was obtained from ART center done on cyflow counter and BD facs count. All patients were classified as those having AIDS ($\text{CD4} \leq 200/\mu\text{L}$) and Non AIDS ($\text{CD4} > 200/\mu\text{L}$), according to NACO criteria. Hematologic parameters were obtained by Sysmex KX-21 automated cell counter. Anaemia was defined as clinically evident anaemia i.e. haemoglobin level ≤ 10 gm/dl. Bone marrow aspirate was obtained after an informed consent from HIV/AIDS patients with anaemia. Bone marrow was aspirated with the help of Salah's bone marrow aspiration needle from posterior superior iliac spine under local anaesthesia and antiseptic precautions. Slides were prepared from marrow particles and were fixed in absolute methanol. Smears were routinely stained with leishman stain, Perl's Prussian blue stain and acid fast stain. Grading of iron stores on bone marrow aspiration was done as documented by Gale *et al*⁶. Periodic Acid Schiff stain was done in selected cases.

2.1 Statistical analysis

Results were tabulated in Microsoft office excel worksheet and expressed in mean \pm standard deviation for continuously distributed variable and in absolute numbers and percentages for discrete variables. Appropriate standard statistical methods were utilized. Chi-square test and p-value were analyzed. p value of less than 0.05 was considered significant.

3. Results

Our study included 75 HIV/AIDS patients with anaemia having haemoglobin ≤ 10 gm/dl. Baseline hematological parameters showed mean haemoglobin 7.58 ± 2.00 gm/dl. Normocytic normochromic anaemia was the commonest type of anaemia seen in 58.66% patients, followed by microcytic hypochromic anaemia in 28% patients.

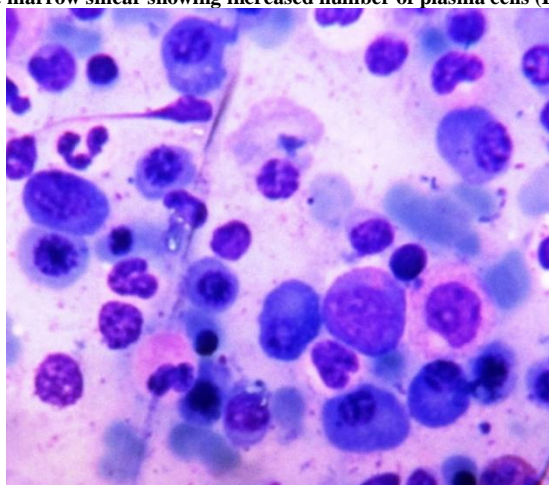
Bone marrow morphology was assessed and analyzed considering features like adequacy, cellularity, myeloid to erythroid ratio, features of erythroid, myeloid and megakaryocytic series, lymphoid cells, plasma cells, macrophages, iron content, presence of abnormal cells, parasites, fungi and acid fast bacilli.

Bone marrow findings are summarized in table 1. Normocellular bone marrow was present in 62.66% patients, followed by hypocellular bone marrow in 21.33% hypercellular bone marrow in 16% patients. Normocellular bone marrow was most common bone marrow cellularity in both AIDS and Non-AIDS patients. Hypocellular bone marrow was more common in AIDS patients (CD4 count $\leq 200/\mu\text{l}$) i.e. 28.94% than Non-AIDS patients (13.54%). Myelodysplasia was an important observation in all the three lineages. Erythroid dysplasia was most common (36%), followed by megakaryocytic series (36%). Dysplasia was seen in 57.89% of AIDS patients as compared to only 42.10% of Non-AIDS patients. Though all three lineages showed higher percentage of dysplasia in AIDS patients, no statistically significant association could be found. Reactive plasmacytosis [Fig. 1] was found in 36% of patients. Increased eosinophilic precursors were seen in 30.66%. Stainable iron in bone marrow was found to be normal in most of the patients i.e. 60%, increased in 16% and decreased in 24% of total cases.

Table No 1. Bone marrow findings

Bone marrow parameters	Number	Percentage
Normocellular	47	62.66
Hypercellular	12	16
Hypocellular	16	21.33
Dyserythropoiesis	51	68
Dysgranulopoiesis	18	24
Dysmegakaryopoiesis	27	36
Increased plasma cells	27	36
Increased eosinophilic precursors	23	30.66
Normal marrow iron	45	60
Increased marrow iron	12	16
Decreased marrow iron	18	24

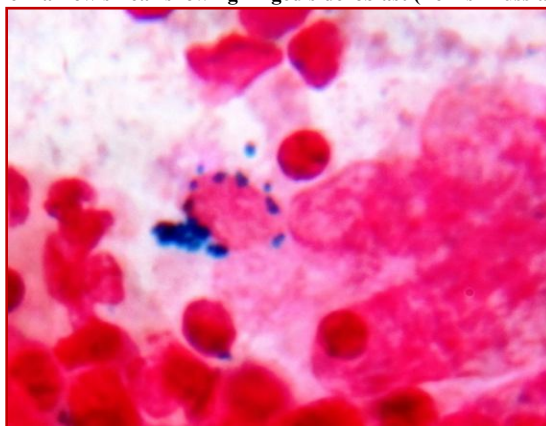
Figure 1- Bone marrow smear showing increased number of plasma cells (Leishman, x400)



Dyserythropoiesis was found in the form of megaloblastoid change, binucleation/multinucleation, cytoplasmic vacuolation, micronormoblastic change and ringed sideroblast [Fig.2][Table 2].

Table 2: Various forms of erythroid dysplasia

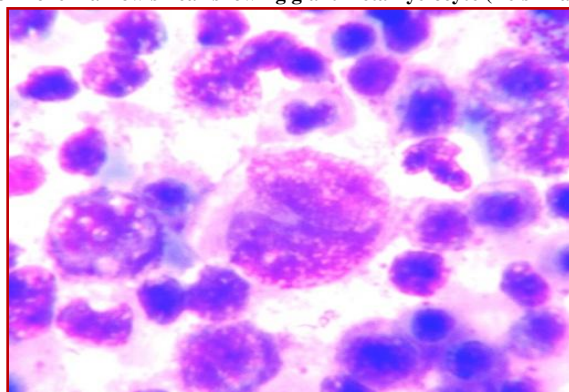
Form of dysplasia	Number (n=51)	Percentage
Megaloblastoid	21	41.17
Binucleation/multinucleation	16	31.37
Cytoplasmic vacuolation	8	15.68
Micronormoblastic	4	7.84
Ringed sideroblast	2	3.92

Figure 2- Bone marrow smear showing ringed sideroblast (Perl's Prussian blue, x1000)

Amongst the patients with dysgranulopoiesis, various dysplastic features seen were nuclear dysmorphism, cytoplasmic vacuolation and giant metamyelocytes [Fig. 3] [Table 3].

Table No. 3: Various forms of myeloid dysplasia

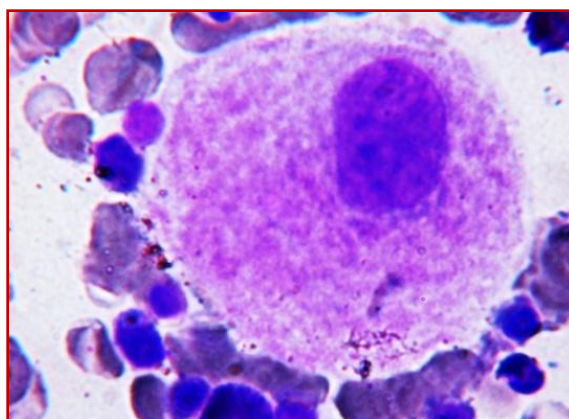
Form of dysplasia	Number (n=18)	Percentage
Nuclear dysmorphism	8	44.44
Giant metamyelocytes	8	44.44
Cytoplasmic vacuolation	2	11.11

Figure 3- Bone marrow smear showing giant metamyelocyte (Leishman, x1000)

In megakaryopoiesis, dysplastic changes seen were hypolobation, hyperlobulation and fragmentation of nuclei [Fig. 4] [Table 4].

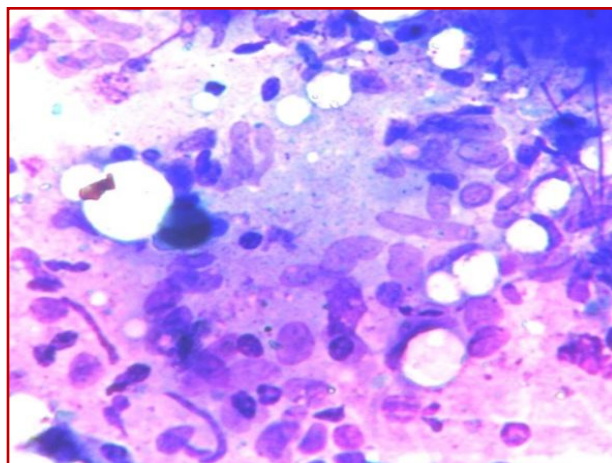
Table No. 4: Various forms of megakaryocytic dysplasia

Forms of dysplasia	Number(n=27)	Percentage
Hypolobation	14	51.85
Hyperlobulation	11	40.74
Fragmentation of nuclei	2	7.40

Figure 4- Bone marrow smear showing hypolobation in megakaryocyte (Leishman, x1000)

Out of total 75 patients, lymphoid cells were found to be normal in 65.73%, decreased in 29.33% and increased in 5.33%. Bone marrow granuloma [Fig. 5] was seen in one case, however Ziehl-Neelsen staining could not reveal acid fast bacilli.

Figure 5- Bone marrow smear showing epithelioid cell granuloma (Leishman, x400)



3. Discussion

Bone marrow abnormalities are seen commonly in HIV infected patients during the course of disease in the form of increased cellularity, dysplasia or granulomatous involvement. The exact mechanism of HIV induced bone marrow changes are not known, however, these are possibly due either to 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.

In the present study, the most common peripheral blood abnormality was normocytic normochromic anaemia. Parinitha *et al*⁷ also reported normocytic normochromic anaemia as most common type seen in 40.4% patients. According to study done by Attili *et al*⁸, Anaemia and neutropenia can be considered as good clinical indicators to predict and assess the underlying immune status.

Bone marrow picture in our study were normocellular in majority of patients, as encountered in other studies^{1,9,10}. Hypocellular bone marrow was detected in 21.33% of patients and was found more common in AIDS than Non-AIDS patients. This finding is in concordance with the study done by Castella *et al*¹⁰ who reported normocellular bone marrow in 67.30%, followed by hypocellular bone marrow in 17.3%. The variation in cellularity in our study from other studies^{2,3} could be explained by the fact reported in literature that a hypocellular bone marrow may be seen in early stage of disease but it is more likely to be normocellular or hypocellular in advanced stage of disease⁹. Our study population is from hospitalized patients, hence more likely to be in advanced stage of disease.

The incidence of myelodysplasia reported in literature showed wide range of variation. In our study we found dysplastic changes in 76% of patients, although, in various studies^{1,11} range varies from 30-90%. 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 large number of patients.

The most common dysplastic change found was dyserythropoiesis, followed by dysmegakaryopoiesis. Our study is in concordance with the study done by Tripathi *et al*¹² and Karcher *et al*¹³. However, some studies^{2,9} reported megakaryocytic and granulocytic series as the most common cell line involved respectively. Lack of concordance with these studies is difficult to explain. Various dyspoietic features seen in erythroid series in our study were megaloblastoid change, bi/multinucleation, cytoplasmic vacuolation, micronormoblast and ringed sideroblasts. Megaloblastoid change seen in HIV related myelodysplasia is unrelated to serum cobalamine and folate levels, or to drug therapy with zidovudine or folate antagonists, although these drugs may accelerate it¹².

Dysplastic changes involving myeloid series, seen in our study include nuclear dysmorphism, giant metamyelocytes, followed by cytoplasmic vacuolation. Various other dysplastic changes involving myeloid series, reported in literature^{1,2,14} also include, hypogranulation and pseudo-pelger-heut anomaly. Although, several of the dysplastic changes noted in HIV infected patients resemble those seen in preleukemic syndromes, myeloid leukemia in AIDS, though reported is very uncommon¹². Direct infection of marrow precursors by HIV may contribute to these defects, although this issue remains controversial. In addition to various dysplastic changes observed in myeloid series we also observed increased number of eosinophilic precursors in 30.66%. Khandekar *et al*¹⁴ also noted increased eosinophilic precursors in bone marrow of 32.14% of HIV infected individuals, similar to our study. Increased eosinophilic precursors could be attributed to reactive changes associated with occult/clinically evident infection. Dysplastic changes involving megakaryocytes found in our study include most frequently hypolobation, followed by hyperlobulation with open nuclear chromatin and cytoplasmic immaturity. Few megakaryocytes also showed presence of fragmented nuclei. Interestingly, we also found emperipolesis in occasional megakaryocytes.

Plasma cells are often strikingly increased in the marrow of HIV infected patients seen in 31-85% of patients^{13,15}. Our patient population had plasmacytosis in 36% of patients. It may represent a physiological response to antigenic stimulation by viruses or other infective agents or may be secondary to dysregulated B-cell proliferation due to HIV.

Lymphoid cells in our study were found to be normal in majority of patients. They were decreased in AIDS patients more than Non-AIDS patients, however no statistically significant association could be found.

The finding of small granulomata without acid fast bacilli on bone marrow aspiration as seen in one case is of clinical interest. Ziehl-Neelsen stain for acid fast bacilli was negative. Periodic acid Schiff stain was done to rule out opportunistic infections like histoplasmosis, cryptococcosis and candidiasis, however, no specific organism could be found. A similar finding was noted in 2/42 cases by Sitalakshmi *et al*³, both of which were found as coexistent cases of pulmonary tuberculosis with HIV infection. The low incidence of granuloma in our study is justified because other studies in which granuloma was found were by trephine biopsy, whereas our's was aspiration study where it is a rare finding.

Perl's Prussian blue stain was performed in all cases to evaluate iron content of the bone marrow. We found adequate stainable iron in majority of cases (60%), decreased in 24%, and increased in 16%. Our findings are in tune with most other studies^{2,14}. Lack of concordance with few other studies^{10,13} could be attributable to higher incidence of reticuloendothelial blockade in their patients which in turn can be due to advanced stage of infection at the time of presentation.

4. Conclusion

Our purpose in performing this study was to observe various morphological changes in HIV infected anaemic individuals and also to evaluate stainable iron in the bone marrow. We observed various myelodysplastic changes in all the cell lineages although erythroid series was most frequently involved. We also observed that these dysplastic changes increase with the progression of the disease. Evaluation of iron stores showed adequate stainable iron in most of cases consistent with anaemia of chronic disease. HIV infection should be included in differential diagnosis of patients with secondary myelodysplasia.

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