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Bone Marrow Aspiration Findings in HIV Positive Patients and Correlation with CD4 Count.

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ABSTRACT

Background: Bone marrow abnormalities are common in HIV infected individuals and patients with AIDS. Most of the bone marrow abnormalities associated with HIV infection appear to be related directly to the infection or its complications and not to therapeutic intervention. Bone marrow study is an important investigation in HIV infected patients with peripheral hematological abnormalities. The primary objective was to study bone marrow aspiration findings in HIV positive patients and their correlation with CD4 count. **Methods:** The interventional, crossectional and prospective study was conducted in Department of Pathology, Government Medical College and Rajindra Hospital Patiala on 100 HIV positive patients, during a period of 2 years. Hematological investigations including complete hemogram, peripheral blood film and bone marrow aspiration was done. Absolute CD4 counts were done. Correlation of various bone marrow abnormalities was done with CD4 count. **Results:** Bone marrow revealed normocellular marrow in majority of cases, followed by hypocellular. Overall prevalence of myelodysplasia was 78%. Dyserythropoiesis was most common dysplastic change (62%) followed by dysmegakaryopoies (36%), dysgranulopoiesis (25%). Reactive plasmacytosis was seen in 44% cases in the range from 6-20%. Increased lymphocytes seen in 9% cases. There was seen significant correlation between myelodysplasia and CD4 count. **Conclusion:** Bone marrow abnormalities are common in HIV infected individuals and patients with AIDS.So HIV infection should be considered in the differential diagnosis of patients with secondary myelodysplasia or unexplained bone marrow changes.

Keywords: Bone marrow, CD4 count, HIV, Myelodysplasia

INTRODUCTION

Bone marrow abnormalities are found at all stages of HIV disease, increases in frequency as disease progresses. Infection of marrow mesenchymal stem cells with HIV has been incriminated as an important factor causing bone marrow defects. 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 and megakaryocytic colonies in most patients with AIDS.[1] In addition, there is now ample evidence that CD34 progenitor cells from normal bone marrow and fetal hepatic hematopoietic

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Cellular Abnormalities

Dysplasia

A remarkable degree of dysplasia has been noted in the myeloid, erythroid and platelet precursors. Dyserythropoiesis, although initially reported less often than myelodysplasia, it is now increasingly recognized.[2]

Cellularity

The true marrow cellularity is better appreciated on trephine biopsy, which is typically normocellular to hypercellular even in the setting of peripheral cytopenias in a majority of patients.[3]

Plasma Cells Abnormalities

Plasma cells are often strikingly increased in the marrow of HIV-infected patients seen in 31-85% of patients.[2,4] These 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.

Other Abnormalities

Additional cellular abnormalities of bone marrow include increased numbers of histiocytes, few

showing haemophagocytosis, non caseating granulomas and the presence of "loose granulomas" consisting of aggregated histiocytes, lymphocytes and plasma cells.[5,6]

Abnormalities In Bone Marrow Matrix

Abnormalities in the bone marrow matrix are frequently seen and include increased reticulin or fibrosis and serous atrophy or "gelatinous transformation".[4,5,7]

Infectious agents reported to involve the bone marrow in patients with AIDS are Mycobacterium avium complex, Mycobacterium tuberculosis, Mycobacterium xenopi and kansasi, Histoplasma, Cryptococcus, Toxoplasma, Cytomegalovirus, Leishmania, Pneumocystis carinii, Disseminated cat scratch, Parvovirus B 19.

MATERIALS AND METHODS

The study was conducted on 100 HIV positive patientsin Department of Pathology, during a period of 2 years. The study proposal and procedures were approved by the ethical committee of Government Medical College, Patiala. All the patients who were known cases of HIV or newly diagnosed cases of HIV. (In whom bone marrow aspiration was indicated and suspected cases of infections and hematological malignancies were also included in the study.

Patients who were on antiretroviral treatment, pregnant women, new born babies who were born to HIV positive mothers, patients of malignancy not related to HIV disease and patients receiving chemotherapy were excluded from the study.These patients were divided according to CDC criteria based on CD4 counts. Those having CD4 counts <200/µl considered as AIDS and those having CD4 counts >200/µl considered as non- AIDS.

Detailed history was taken. Physical examination was done. Hematological investigations including complete hemogram (hemoglobin, total leucocyte count, differential leucocyte count, platelet count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, total red blood cell count, reticulocyte count, absolute neutrophil, lymphocyte, monocyte, eosinophil and basophill counts), peripheral blood film were done. Bone marrow aspiration was done. Absolute CD4 counts were done for their correlation with bone marrow abnormalities.

Procedure

Hematological parameters were analysed in haematology auto analyserSysmex XP-100 which analyses using three detector blocks. White blood cells (WBC) count, red blood cell (RBC) count, platelets were measured using direct current detection method. CD4 lymphocyte count was done in BD FACS count flow cytometer, an automated multicolour system that perfoms both analysis and sorting using dual lasers 488 nm air cooled argon-ion laser and 635 nm red diode laser.

Bone marrow examination was performed for indication of anemia, leucopenia, pancytopenia and thromobocytopenia. Posterior superior iliac spine was chosen as site for bone marrow aspiration because of large marrow space and least painful site. The aspiration was done by Salah's bone marrow aspiration needle.

The Centers for Disease Control and Prevention (CDC) criteria were used to classify the study population into three categories based on CD4 counts: Stage 1 (CD4>500 cells/µl), stage 2 (CD4 between 200 and 499 cells/µl) and stage 3 (CD4<200 cells/µl).Bone marrow findings were then correlated with the CD4 cell counts being divided into two groups:1) less than 200 cells/µl (AIDS) 2) more than 200 cells/µl (non-AIDS).

Statistical tests used included mean, standard deviation, chi-square test (x2), and ttest.Descriptive statistics were applied; p value less than 0.05 was considered statistically significant.

RESULTS

Out of 100 patients , majority of patients were in the age range of 21- 40 years and mean age was 37.33.+11.797 with 62% males and 38% females. There was male preponderance with male: female ratio1.6:1.The overall prevalence of anemia, leucopenia and thrombocytopenia was 88%, 22%, 21% respectively. Bone marrow revealed normocellular marrow (62%) in majority of cases, followed by hypocellular (23%) and hypercellular (15%). There was no significant correlation between cellularity and CD4 count. Normoblastic reaction was the most common finding seen in 55% followed by megaloblastic reaction seen in 35%. There was no significant correlation between eryhroid reaction and CD4 count. Overall prevalence of myelodysplasia was 78%.The most common dysplastic change found in bone marrow aspiration smear was dyserythropoiesis seen in 62% cases followed by dysmegakaryopoiesis seen in 36%, dysgranulopoiesis was least commonly noted in 25% cases. There was seen significant correlation between myelodysplasia and CD4 count. Erythroid series was normal in majority of patient but erythroid suppression and erythroid hyperplasia was also seen. But these finding did not show any correlation with CD4 Count. Granulocytic series was normal in majority of patient but granulocytic suppression and granulocytic hyperplasia was also seen. There was seen significant correlation between granulocytic series and CD4 count. Megakryocytic series was normal in majority of patient but megakryocytic suppression and megakaryocytic hyperplasia was also seen. But these did not show any significant correlation with CD4 count. Reactive plasmacytosis

was seen in 44% cases in the range from 6-20%. But these did not show any significant correlation with CD4 count. Increased lymphocytes seen in bone marrow aspiration smear in 9% cases. But these did not show any significant correlation with CD4 count. Marrow iron was adequate in 90% of patients. Gelatinous transformation of bone marrow was seen in 3% cases

Table 1: Correlation of cellularity with CD4 count.

Cellularity	CD4 count		Total
	<200	>200	
Normocellular	34 (58.6%)	28 (66.7%)	62 (62%)
Hypocellular	16 (27.6%)	7 (16.7%)	23 (23%)
Hypercellular	8 (13.8%)	7 (16.7%)	15 (15%)
Total	58 (100%)	42 (100%)	100 (100%)
Chi square	Df	p value	
1.651	2	0.438	

There was no significant correlation between cellularity and CD4 count.

Table 2: Correlation of Myelodysplasia with CD4 count.

Dysplasia	CD4 count		Total
	<200	>200	
Dysplasia present	50 (86.21%)	28(66.67%)	78(78%)
Dysplasia absent	8(13.79%)	14(33.33%)	22(22%)
Total	58 (100%)	42 (100%)	100 (100%)

There was seen significant correlation between myelodysplasia and CD4 count

Table 3: Correlation of Granulocytic series with CD4 Count

Granulocytic Series	CD4 count		Total
	<200	>200	
Normal	36 (62.1%)	35 (83.3%)	71 (71%)
Suppressed	12 (20.7%)	2 (4.8%)	14 (14%)
Hyperplasia	10 (17.2%)	5 (11.9%)	15 (15%)
Total	58 (100%)	42 (100%)	100 (100%)
Chi square	Df		p value
6.428	2		.040

There was seen significant correlation between myelodysplasia and CD4 count

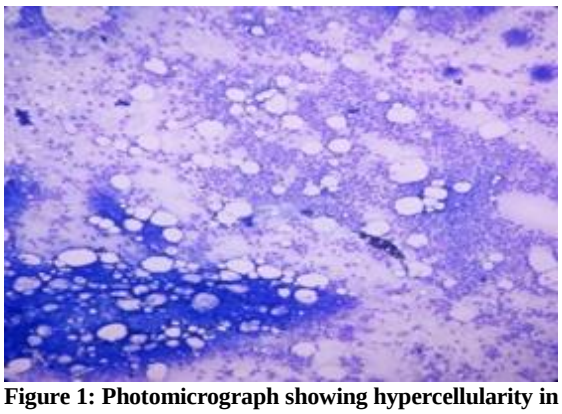

Figure 1: Photomicrograph showing hypercellularity in bone marrow aspiration smear. (100X)

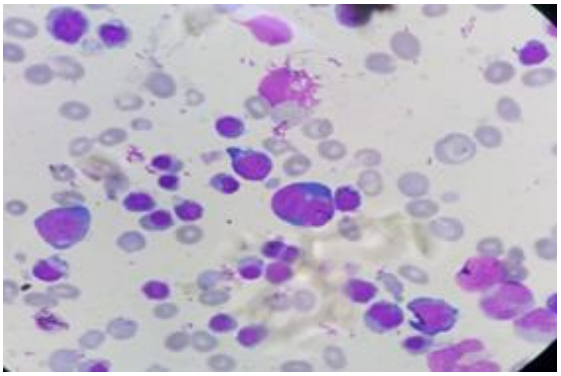

Figure 2: Photomicrograph showing BMA smear with features of Dyserythropoiesis, Megaloblast and Plasma cells. (400X)

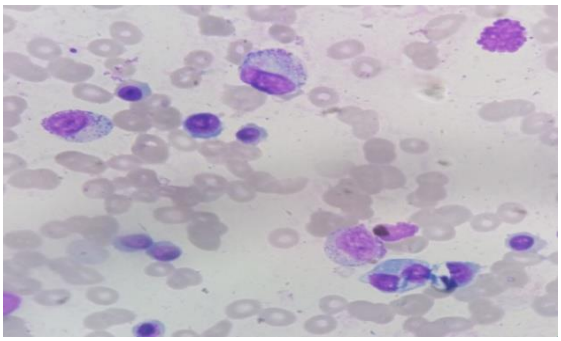

Figure 3: Photomicrograph showing BMA smear with features of Dyserythropoiesis and Reactive changes in Myelocytes. (400X).

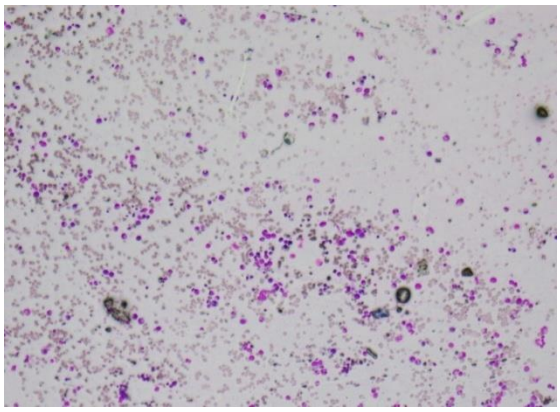

Figure 4: Photomicrograph showing hypocellularity in BMA smear. (100X)

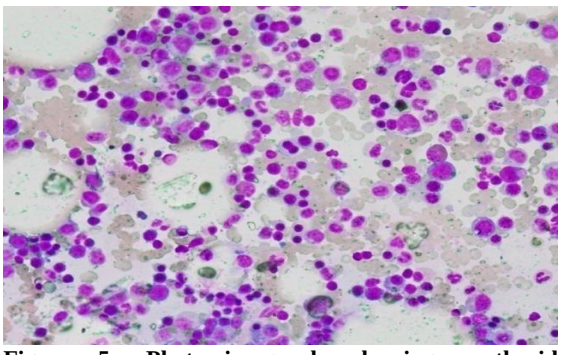

Figure 5: Photomicrograph showing erythroid hyperplasia and features of Dyserythropoiesis in BMA smear. (400X)

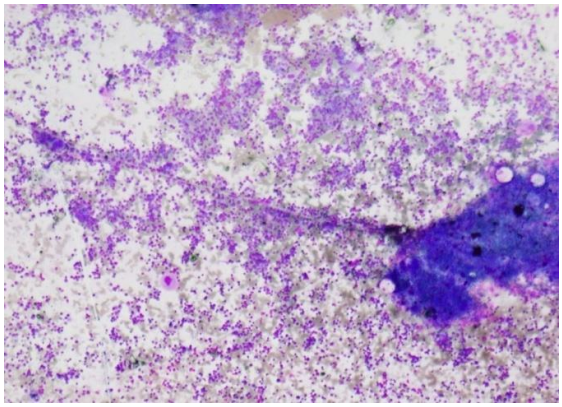

Figure 6: Photomicrograph showing hypercellularity and features of dysmegakaryopoiesis in BMA smear. (100X)

DISCUSSION

Bone marrow abnormalities are seen commonly in HIV infected patients during the course of disease.

Age and sex distribution

In the present study 100 HIV positive patients were recruited as per inclusion and exclusion criteria. Majority of patients were in the age range of 21-40 years and mean age was 37.33.+11.79 with 62% males and 38% females. There was male preponderance with male: female ratio 1.6:1.

These findings were in concordance with study done by Parinitha et al in (2012),[8] study by Kotwal et al in 2013 and study by Rehman et al in 2014.[9,10]

Table 4: Correlation of cellularity with CD4 cell count - comparison with other studies

Authors	Normocellular CD4 count		Hypocellular CD4 Count		Hypercellular CD4 Count	
	<200	>200	<200	>200	<200	>200
Tripathi et al[11](2005)	74.55%	78.95%	7.27%	5.26%	18.18%	15.79%
Dhruve et al [12] (2013)	79.68%	79.06%	12.15%	13.95%	7.8%	6.97%
Present study	58.6 %	66.7%	27.6%	16.7%	13.8%	16.7%

Table 5: Comparing myelodysplasia with other studies

Authors	Dysplasia	Erythroid dysplasia	Granulocytic dysplasia	Megakaryocytic dysplasia
Karcher et al[2] (1991)	69%	56%	18%	31%
Mittal et al [14] (2014)	76%	68%	24%	36%
Present study	78%	62 %	25%	36 %

In the present study out of 100 patients, bone marrow was normocellularin 58.6%, hypocellular in 27.6%, hypercellular in 13.8% in AIDS patients (CD4 count below 200). Among AIDS patients (CD4 count above 200) bone marrow was normocellular in 66.7%, hypocellular in 16.7%and hypercellular in 16.7% cases, this is in concordance with studies shown above [Table 4].

We did not find any statistically significant correlation between cellularity and CD4 count. This is in concordance with study done by Pande et al (2011).[13]

In the present study myelodysplasia was seen in bone marrow aspiration smears of 78% cases, this is in concordance with study by Karcher et al (1991) in which dysplasia is seen in 69% cases and Mittal et al (2014) in which dysplasia was seen in 76% cases.[2,14] It was found that some patients had shown dysplasia in bilineage as well as trilineage. Most common series affected was erthroid series. Erythroid dysplasia was seen in 62% followed by megakaryocytic dysplasia, seen in 36% cases and granulocytic dysplasia was seen in 25% cases, this is similar with studies done by Karcher et al (1991) showing erythroid, megakaryocytic and granulocytic dysplasia in decreasing order (56%, 31%and 18%respectively).[2] Mittal et al (2014) observed that erythroid dysplasia was most common (68%),[14] followed by megakaryocytic dysplasia (36%) and granulocytic series (24%).

Correlation of myelodysplasia with CD4 count

In our study, it was seen that myelodysplasia was more common in AIDS patients (CD4 count below 200), we found statistically significant association between myelodysplasia and CD4 Count. This is in concordance with study done by Tripathi et al (2005).[11] However in our study, although all three lineages showed higher percentage of dysplasia in AIDS patients (CD4 lymphocyte counts below 200), statistically significant association found between granulocytic dysplasia and CD4 count.

Table 6: Comparing granulocytic series with other studies

Authors	Normal	Suppressed	Hyperplasia
Pande et al[13] (2011)	73.9%	15.22%	10.87%
Nirmla et al [15] (2015)	63.63%	23.33%	
Present Study	71%	14%	15%

In the present study in 71% patients granulocytic series was normal. Granulocytic suppression was seen in 14% cases and granulocytic hyperplasia in 15%. This is in concordance with study done by Pande et al (2011) which showed normal granulocytic series in 73.9% cases,[13] granulocytic suppression in 15.22% and granulocytic hyperplasia in 10.87%. Study done by Nirmla et al (2015) showed normal granulocytic series in 63.63% cases while granulocytic suppression in 23.33%.[15]

Correlation of granulocytic series with CD4 count - comparison with other studies

In the present study suppressed granuopoiesis was seen in 20.7% in AIDS Group (CD4 Count below 200) and in 4.8% in non AIDS (CD4 Count above 200). Hyperplasticgranulopoiesis was seen in 17.2 %

in AIDS Group (CD4 Count below 200) and in 11.9% in non AIDS (CD4 Count above 200).This is comparable with study done by Pande et al (2011) showing suppressed granulopoiesis in 14.24% in AIDS Group (CD4 Count below 200) and in 18.18% in non AIDS (CD4 Count above 200).[13] Hyperplastic granulopoiesis was seen in 11.43 % in AIDS Group (CD4 Count below 200) and in 9.09% in NON AIDS (CD4 Count above 200). We could estabilishstatistically significant correlation between granulocytic series and CD4 count.

CONCLUSION

Bone marrow abnormalities are common in HIV infected individuals and patients with AIDS. Most of the bone marrow abnormalities associated with HIV infection appear to be related directly to the infection or its complications and not to therapeutic intervention. Bone marrow study is an important investigation in HIV infected patients with peripheral hematological abnormalities. In majority the marrow were normocellular, although in a significant proportion it was found to be hypocellular. Reactive plasmacytosis was detected in 44% of patients. Myelodysplasia was seen in 78% cases. Dysplastic changes involving isolated cell lines ranged from 25% to 62%, dyserythopoiesis being the most common. Myelodysplasia was seen more common in AIDS than in non AIDS and was significantly associated with lower CD4 count. So HIV infection should be considered in the differential diagnosis of patients with secondary myelodysplasia or unexplained bone marrow changes.

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