A STUDY OF BONE MARROW CHANGES IN HUMAN IMMUNODEFICIENCY VIRUS INFECTION

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ABSTRACT: INTRODUCTION: The haematological abnormalities in HIV infected patients is complex and is more commonly associated with anaemia. This study was conducted to evaluate the incidence of various haematological abnormalities occurring in HIV infected individuals and to observe the difference in the haematological bone marrow findings in patients on ART as compared to not on ART. **AIMS:** 1. To various haematological abnormalities in HIV patients. 2. To evaluate the bone marrow findings in the patients. 3. To evaluate the difference in haematological abnormalities in HIV pts on HAART treatment compared to patients not on treatment. **MATERIALS AND METHODS:** A Retrospective study done at department of pathology, Bowring and Lady Curzon hospitals from May 2010 to May 2015 for 5 yrs. 49 HIV positive patients with haematological abnormalities and PUO underwent bone marrow aspiration. The bone marrow smears were air dried and stained with leishman stain & giemsa stain. Trephine biopsy was stained with H & E, Zeihl neelsen stain for AFB was done for selected cases. The bone marrow slides were studied for cellularity, presence of dyshematopoiesis. Patients were classified in to ART and NON ART group. **RESULTS:** 1. Anaemia, leucopenia, thrombocytopenia and pancytopenia were observed in both groups of patients of HIV [On HAART and not on HAART]. 2. Bone marrow in patients not on HAART are more often associated with a hypoplastic/normocellular marrow with reduced counts of the precursor cells with the cytopenias. 3. Bone marrow picture in patients on HAART treatment are associated with a hypercellular marrow with features of dyserythropoiesis and ineffective erythropoiesis as the cause of the cytopenias.

KEYWORDS: Hiv, Bone marrow, Dyserythropoiesis.

INTRODUCTION: The haematological abnormalities in HIV infected patients is complex and is more commonly associated with anaemia.^[1] Uncorrected anaemia results in multisystem disabling symptoms and fatigue, exhaustion, increased risk of HIV dementia poor quality of life and possibly exacerbates poverty in communities with high HIV prevalence.^[2]

HIV infection may lead to anaemia by changes in cytokine production with subsequent effects on haematopoiesis, decreased erythropoietin production, due to treatment with HAART drugs, nutritional deficiency, opportunistic infection, malignancy and myelophthisis of marrow caused by lymphoma.^[2]

Research on haematological complications of HIV disease will lead to effective management of cases and and reduce the morbidity and mortality from this dreaded disease.^[3]

HAART has dramatically reduced morbidity and mortality worldwide however a 3 fold increased rate of mortality within the first 12 months of post HAART initiation.^[4]

This study was conducted to evaluate the incidence of various haematological abnormalities occurring in HIV infected individuals and to observe the differences in the haematological bone marrow findings in patients on HAART as compared to not on HAART.

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AIMS:

1. To various haematological abnormalities in HIV patients.
2. To evaluate the bone marrow findings in the patients.
3. To evaluate the difference in haematological abnormalities in HIV patients on HAART treatment compared to patients not on treatment.

MATERIALS AND METHODS: The study population included 49 HIV positive patients with haematological abnormalities and PUO were included in this study. HIV was diagnosed by ELISA method as per NACO guidelines. The study is retrospective study done at department of pathology, Bowring and Lady Curzon hospitals from May 2010 to May 2015 for 5 yrs.

Bone marrow aspiration was performed as a part of investigation for pyrexia of unknown origin and for haematological abnormalities ie., peripheral pancytopenias, anaemia, and thrombocytopenia. Relevant clinical history, baseline investigation, peripheral smear finding were noted in these patients.

Bone marrow aspiration was done under aseptic precautions from the posterior superior iliac spine. Bone marrow biopsy was done where ever indicated. The bone marrow smears were air dried and stained with leishman stain & giemsa stain. Trephine biopsy was stained with H&E, Zeihl neelsen stain for AFB was done for selected cases

The bone marrow slides were studied for cellularity, presence of dyshematopoiesis.

Patients were classified in to HAART and NONHAART group.

RESULTS: Age group of the patients ranged from 19 yrs to 60 yrs. There was slight male preponderance with 29 males (59.1) and 20 females (40.81). 27 patients were on HAART and 22 patients were NONHAART.

Out of the 49 cases, 5 cases cellularity could not be assessed as it was diluted with blood.

In HAART patient's bone marrow was normocellular in 5 cases (18.51%), hypercellular in 13 cases (48.14%) and hypocellular in 7 cases (25.92). In NONHAART cases normocellularity was seen in 10 cases (45.45), hypercellularity in 4 (18.18%) and was hypocellular in 5 cases (22.72). [TABLE 1].

In HAART patients, 12 (44.44%) showed normoblastic pattern of maturation, 8 (47%) patients showed megaloblastic pattern of maturation. Dyserythropoiesis was seen in the form of nuclear budding, lobulated nucleus, binucleate, fragmented nuclei and megaloblastic changes was seen in 10 (30%) cases of HAART patients. In NON HAART patients 8 (36.36%) patients showed normoblastic pattern of maturation, 7 (31.8%) showed megaloblastic pattern of maturation and two patients showed micronormoblastic pattern of maturation. Dyserythropoiesis was seen in 8 (36.36%) Cases of NON ART patients.

In HAART patients, 12 (44.44%) showed normal pattern of maturation in myeloid series, 9 (33.33%) patients had dysmyelopoiesis which included abnormal shaped nuclei, folded, cleaved nuclei, giant metamyelocyte, myelocyte, pseudo pelger heut anomaly, vacuolisation of the cells and maturation arrest. 6 (22.22%) patients showed decrease in myeloid series. [Fig. 1].

In NON HAART patients, 14 (63.63%) patients showed normal pattern of maturation in myeloid series, 5 (23.33%) patients had dysmyelopoiesis and 3 patients (13%) showed reduced count.

In HAART patients 18 (66.66) showed normal megakaryocyte count, 4 (14.81) patients showed reduced count and dysmegakaryopoiesis in 4 patients which included hypolobated and

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bizarre shaped nucleus in megakaryocyte. [Fig. 2]. In NONHAART patients 16(72.72) cases showed normal count, 3(13.63%) case showed reduced count and 3(13.63%) cases showed dysmegakaryopoiesis.

In art patients 6 patients showed increase in plasma cells and in non-ART cases 8 patients showed increase in plasma cells.

2 patients showed opportunistic infection, one with mycobacterium tuberculosis infection and other case showed mycobacterium avium intracellulare complex. The granulomas in MAC infection are small illformed with proliferation of foamy macrophages. On ZN staining of bone marrow aspiration smears these macrophages were heavily laden with AFB positive bacilli. These bacteria are typically longer more curved and more coarsely beaded than the tubercular bacilli and are PAS positive. They are present in large numbers. [Fig. 3]

Non hodgkins lymphoma was seen in one patient, involving the bone marrow of the large cell type. [Fig. 4]

DISCUSSION: In the present study of HIV patients 27 were on HAART and 22 patients were not on HAART. Most patients are in the group of 31-40 yrs. The most common haematological abnormality was pancytopenia in both groups of patients. Pancytopenia was seen more often in NON HAART (59.09) than in HAART (44.44). Anemia and bicytopenia was more commonly seen in HAART group. Hypercellular marrow in the face of peripheral pancytopenia is a very common finding in HIV disease and is likely to reflect myeloid dysplasia and ineffective erythropoiesis.⁽⁵⁾

The HIV associated hematopoietic dysfunction is that after myelosuppression, bone marrow recovery, a process known to be mediated in part by production of stromal cell derived hematopoietic growth factor is impaired.^[6] The stromal cells may produce inhibitory factors which suppress clonogenic potential of mesenchymal stem cells.^[5] The infection of stromal cells could alter the haematopoietic microenvironment and in turn affect development of uninfected progenitor cells.^[6]

In vitro studies have shown that HIV can directly infect the hematopoietic and mesenchymal cells and can influence their activity.^[7]

The marrow morphological characteristics are associated with peripheral blood cytopenias of one or more lineage: anemia, granulocytopenia or thrombocytopenia.

The pathophysiology of these observed deficiencies are still unclear, although several mechanisms have been postulated as possible explanations for the hematological features in AIDS patients. It is not yet known whether HIV infection of marrow stromal cells may play a role in altering hematopoiesis, however HIV infection of microvascular endothelial cells impaired their capacity to respond to IL-1- α -induced release of IL-6 and G-CSF. Exaggerated expression of tumour necrosis factor, Interleukin-1, and transforming growth factor- α 1 by HIV infected monocytoïd cells further impedes hematopoiesis.⁽⁵⁾

An Indian study revealed anaemia as the most common haematological abnormality which was normocytic normochromic type in 61% of patients.^[5]

The bone marrow changes include varying degree of dysplasia in one or more cell lines most common being erythroid dysplasia seen in over 50% of infected patients which may mimic MYELODYSPLASTIC SYNDROMES.^[5]

Several studies have shown that prevalence of anemia is high in HIV and AIDS. Several observational studies have also reported a higher mortality in HIV infected patients from low

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haemoglobin levels even after adjusting for CD4 cell count and viral load.^[8] The use of highly active antiretroviral therapy is associated with an increase in haemoglobin concentrations and decrease in the prevalence of anemia.^[8]

The HAART therapy as per national guidelines in India includes fixed dose combination of 2 NRTI [Zidovudine or stavudine PLUS Lamuvidine] and one NNRTI [Niveripine/Efavirenz]. Zidovudine was prescribed if haemoglobin was 8gm/dl and Stavudine if less than 8gm/dl.^[9]

Zidovudine is associated with mitochondrial and haematological toxicity leading to bone marrow aplasia and cytopenias. It also exhibits cytotoxicity to the myeloid and erythroid precursors. This haematological toxicity is observed in most of the patients within 3 to 6 months and is reversible therefore these Patients should be closely monitored for development of bone marrow toxicity. These patients develop normocytic normochromic anemia in 50% and remaining showed macrocytic anemia.^[8]

Zidovudine has a broad myelosuppressive effect *in vivo* and *in vitro*. Its mechanism of induction of anemia is possibly related to reduction of globin mRNA synthesis. Recipients of zidovudine are more likely to experience anemia and neutropenia than stavudine.^[2]

The severity of anemia in HIV patients depends on the clinical and immunologic stage of the disease, worsening in the presence of most opportunistic infection or low CD4 cell count and is highly prevalent in patients with low BMI.^[2]

The studies have found that HAART was an effective treatment for anemia of HIV infection except for the well-known suppressive potential in a few cases.^[2]

Increased incidence of anemia, thrombocytopenia and pancytopenia was noted in ART cases as compared to non-art cases. Dysplasia of marrow cells have been reported in myeloid, erythroid and megakaryocytic lineages which were noted in our study also. Dyserythropoiesis was noted in both art and non-art groups to the same degree in the present study i.e., 37% of cases. Megaloblastic changes were seen up to 30% of the cases. Micronormoblastic changes were seen in 2 % of non-art cases.

In myeloid series dysmyelopoiesis and reduction in myelopoiesis was noted in both the groups. Higher incidence of dysplasia is seen in advanced disease is probably due to increased HIV RNA load, cytokine mediated effect of disease and drug related changes.^[10]

However more degree and number of HAART cases showed these changes along with dysgranulopoiesis. One case of leucopenia showed maturation arrest of myeloid precursors with reduced mature neutrophils and band forms in the marrow. In a study granulocytic series was most commonly associated with evidence of dysplasia as compared to other cells.^[11]

Thrombocytopenia cases were associated with reduced number of megakaryocytes, dysmegakaryopoiesis with a slight increase in cases on ART.

Plasmacytosis was seen in both groups of HIV patients with increased incidence of plasmacytosis in non-art patients. Plasma cells are often increased in the marrow of HIV infected patients.^[12] These may represent a physiological response to antigenic stimulation by viruses or other infective agent, or may be secondary to dysregulated B cell proliferation due to HIV itself.⁽³⁾

One case of atypical mycobacterial infection with mycobacterium avium intracellulare complex was seen in our study. These atypical mycobacterial infections occur usually late in the disease course when CD4 count is very low.^[13] Bone marrow study is a cost effective way of identifying granulomas in opportunistic infections where culture facilities are not available.^[14]

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PANCYTOPENIA is common and attributable to suppression of bone marrow function and in part to haemophagocytosis.

One case of Nonhodgkins lymphoma of diffuse large B-cell type involving the bone marrow was seen in this study. Diffuse large B cell lymphoma constitutes 65% of AIDS related NHL. They occur in very advanced HIV infection when CD4 count is very low. Bone marrow infiltration can occur but is much less frequent than in burkitt and burkitt like lymphomas.^[15]

Diagnostic confusion with myeloproliferative disorders, myelodysplastic syndromes and Tcell lymphomas can occur if the cytological and histological features typical of HIV are not recognised.^[15]

Other features noted in bone marrow in HIV patients include numerous bare megakaryocyte nuclei, polymorphic lymphoid aggregates, gelatinous degeneration, detached nuclear fragments in granulocytes and giant metamyelocytes in the absence of megaloblastosis.^[15]

CONCLUSIONS:

1. Anaemia, leucopenia, thrombocytopenia and pancytopenia were observed in both groups of patients of HIV [on HAART and not on HAART].
2. Bone marrow in patients not on HAART are more often associated with a normocellular/hypoplastic marrow (68.17%) with reduced counts of the precursor cells with the cytopenias.
3. Bone marrow picture in patients on HAART treatment are associated with a hypercellular marrow (48.18%) with features of dyserythropoiesis and ineffective erythropoiesis as the cause of the cytopenias.

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	PARAMETER	ART	NON ART
PERIPHERAL SMEAR FINDING	Anemia	12(44.44%)	4(18.18%)
	Bicytopenia	3(14.81%)	3(13.63%)
	Pancytopenia	12(44.44%)	14(63.63%)
	Leucopenia	00	01(4.54%)
CELLULARITY	Hypercellular	13(48.14%)	4(18.18%)
	Normocellular	5(18.51%)	10(45.45%)
	Hypocellular	7(25.92%)	5(22.72%)
	Cannot Be Assesed	2(7.4%)	3(13.6%)
ERYTHROID SERIES	MEGALOBLASTIC	8(29.62%)	7(31.81%)
	NORMOBLASTIC	12(44.4%)	8(36.6%)
	MICRONORMOBLASTIC	NIL(0)	2(9.09%)
	DYSERYTHROPOEISIS	10(36.36%)	8(36.36%)
MYELOID SERIES	NORMAL	12(45.1%)	14(64.1%)
	REDUCED	6(22.1%)	3(13.1%)
	DYSMYELOPOEISIS	9(33.1%)	5(23.1%)
Megakaryocytes	NORMAL	18(66.1%)	16(66%)
	REDUCED	5(19%)	3(14%)
	DYSMEGAKARYOPOEISIS	4(14.81%)	3(13%)

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Plasma cell			
	Normal	21(77.77)	14(63.63)
	increased	6(25.92)	8(36.36)
Opportunistic infection			
	Mycobacterial	01	nil
	Atypical Mycobacterial(MAC)	nil	01
	Nonhodgkins	00	01
Table 1: Comparison of the various bone marrow abnormalities detected in HAART and non HAART cases			

Fig. 1: Bone marrow aspiration showing myeloid series , myelocytes and metamyelocytes with features of dysmyelopoiesis. Giemsa stain ,100x.

Fig. 1

Fig. 2: Bone marrow aspiration showing megakaryocytes with crowding and bare nuclei. Giemsa stain , 100x.

Fig. 2

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Fig. 3: Bone marrow aspiration, zeil neelsen stain showing plenty of intracellular acid fast bacilli with beaded appearance. ZN stain, 100x.

Fig. 3

Fig. 4: Bone marrow biopsy showing non-Hodgkin's lymphoma composed of large lymphoma cells infiltrating the BM .H & E stain, 40x.

Fig. 4

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