

## STUDY OF HAEMATOLOGICAL ABNORMALITIES IN HIV PATIENTS AND CORRELATION WITH BONE MARROW CHANGES AT A TERTIARY CARE CENTRE

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### ABSTRACT

#### BACKGROUND

HIV infection is a global pandemic with cases reported from virtually every country. In 2016 India had 80,000 (62,000 - 100,000) new HIV infections and 62,000 (43,000 - 91,000) AIDS-related deaths. There were 2,100,000 (1,700,000 - 2,600,000) people living with HIV in 2016, among whom 49% (40% - 61%) were accessing antiretroviral therapy.<sup>(1)</sup> India stands third in HIV epidemic in the world<sup>(2)</sup> with 2.1 million people living with the virus.<sup>(2)</sup> Epi disorders of haematopoietic system including anaemia, leukopenia, and/ or thrombocytopenia are common throughout the course of HIV infection.<sup>(3)</sup>

The objectives of the study are-

1. To review and describe bone marrow abnormalities and associated peripheral abnormalities in HIV/ AIDS patients.
2. To establish the prevalence of trilineage cytopenia.
3. To determine presence of secondary malignancies.

#### MATERIALS AND METHODS

A prospective descriptive clinical study with 104 HIV positive patients consisting of 39 AIDS and 65 non-AIDS patients were undertaken to study haematological abnormalities in HIV patients and correlation with bone marrow changes of tertiary care centre.

#### CONCLUSION

Peripheral and bone marrow abnormalities are common in HIV related disease and has got significant impact on clinical outcomes and quality of life.

- HIV is prevalent more in males as compared to females in sexually active age group of 20 - 49 yrs.
- Bone marrow was hypocellular, five times more common in AIDS than in non-AIDS.
- Dysplasia is common in AIDS more than non-AIDS.

#### RESULTS

Total no. of patients included in the study were 104, out of which 66 were males and 38 were females with sex ratio (M: F= 1.73: 1). These 104 patients were examined. Their clinical and haematological parameters were analysed.

#### KEY WORDS

AIDS- Acquired Immunodeficiency Syndrome, HIV- Human Immunodeficiency Virus, CD- Cluster of Differentiation.

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#### BACKGROUND

Clinically significant haematologic abnormalities are common in people affected with HIV infection. Multiple mechanisms have been described as impaired haematopoiesis immune-mediated cytopenias and altered coagulation mechanisms, out of which most of the abnormalities are caused by HIV infection, sequelae of HIV related opportunistic infection or malignancy.

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#### Some of the Mechanisms that may be Operative are-

1. A direct suppressive effect of HIV on haematopoietic progenitors or marrow stromal cells, autoimmune process.
2. Infiltration of the bone marrow.
3. Ineffective erythropoiesis.
4. Nutritional deficiencies.
5. Suppression of erythropoietin.
6. Cytokine-mediated suppression.
7. Drug toxicity.

In general, haematological abnormalities progress in frequency and severity with progression of infection from asymptomatic HIV carrier state to later asymptomatic stages of the disease.<sup>(4)</sup>

Anaemia	Thrombocytopenia	Leucopenia
<ul style="list-style-type: none"> <li>▪ HIV infection per se<sup>(3)</sup></li> <li>▪ Opportunistic infections-MAC Parvo-B19<sup>(1)</sup>, Histoplasma etc.</li> <li>▪ Drugs ART TMP/SMZ<sup>(3)</sup> dapsone etc.</li> <li>▪ Neoplasma Lymphoma<sup>(3)</sup> Leukaemia</li> <li>▪ Thrombotic</li> <li>▪ Megaloblastic anaemia</li> <li>▪ Autoimmune haemolytic anaemia</li> <li>▪ Pre-existing or co-existing conditions etc.</li> </ul>	<ul style="list-style-type: none"> <li>▪ HIV- associated immune destruction</li> <li>▪ HIV-associated decreased production</li> <li>▪ Adverse drug reaction</li> <li>▪ Thrombotic Thrombocytopenic purpura</li> <li>▪ Hypersplenism</li> <li>▪ Marrow inflammation</li> </ul>	<ul style="list-style-type: none"> <li>● HIV infection per se</li> <li>● Marrow infiltration with tumour or opportunistic infection</li> <li>● Drugs: ART, TMP/SMZ<sup>(3)</sup> dapsone etc.</li> <li>● Neutropenia due to HIV-related decreased production</li> <li>● Anti-neutrophil antibodies</li> </ul>

**Table 1. Causes of Cytopenias in HIV Infection and AIDS<sup>(5)</sup>**

**Morphologic and Histologic Abnormalities of Peripheral Blood and Bone Marrow in Patients with HIV Infection-Peripheral Blood**

**1. Red Cells**

- Normochromic normocytic anaemia.
- Anisocytosis and poikilocytosis<sup>(6)</sup>
- Rouleaux
- Schistocytes
- Reticulocytopenia
- Basophilic stippling
- Macrocytosis (During Zidovudine Therapy)

**2. Neutrophils**

- Enlarged
- Shift to left
- Hyposegmented with Pelger-Huet forms<sup>(7)</sup>

**3. Other**

- Plasmacytoid lymphocytes.
- Large vacuolated monocytes.

4. Bone marrow changes.
5. Marrow hypercellularity.
6. Dyserythropoiesis.
7. Erythroid hypoplasia.

**Objectives**

1. To review and describe bone marrow abnormalities and associated peripheral abnormalities in HIV/ AIDS patients.
2. To establish the prevalence of trilineage cytopenia.
3. To determine presence of secondary malignancies.

**MATERIALS AND METHODS**

**Study Design**

A prospective descriptive clinical study with 104 HIV-positive patients consisting of 39 AIDS patients and 65 non-AIDS patients were undertaken to study of haematological abnormality in HIV patients and correlation with bone marrow changes at tertiary care centre.\*

**Source of Data**

Patients detected to be HIV positive as per WHO criteria attending the Department of Medicine, at Tertiary Care Centre with peripheral haematological abnormalities on complete haemogram.

**Inclusion Criteria**

Patients tested positive for HIV as per NACO guidelines (age above 12 yrs.) and having anyone on the following haematological abnormalities on peripheral smear-

- Anaemia (Hb < 10.0 g%)
- Leucopenia (Total Leukocyte Count < 4,000 cells/uL)
- Thrombocytopenia (Platelet count < 1 lakh/mm<sup>3</sup>)
- Pancytopenia
- Patients given written informed and valid consent.

**Exclusion Criteria**

Patients with any of the following-

- Patients of malignancy not related to HIV diseases.
- Patients receiving chemotherapy or radiotherapy.
- Patients of Hepatitis B and Hepatitis C viral infections.
- Chronic liver diseases (other than explained by HIV).
- Renal diseases (other than explained by HIV).
- Patients on anti-retroviral therapy.

**Method of Collection of Data**

- After pre-test counselling, blood samples were tested for anti-HIV antibodies by ELISA screening test.
- A detailed clinical history and examination was done and information related to each patient was filled in proforma. Complete haemogram and CD<sub>4</sub> + cell counts were done in all patients. Patients were divided into 2 groups based on their CD<sub>4</sub> + counts as AIDS (CD<sub>4</sub> + <200/uL) and non-AIDS (CD<sub>4</sub> + > 200/uL).
- After taking written informed consent from patients eligible for this study, a bone marrow aspiration and biopsy from posterior-superior iliac spine was done under aseptic precautions using Salah needle and Jamshidi needle (11 or 13 gauge size) for aspiration and biopsy respectively. Peripheral smear and bone marrow examination findings were correlated with CD<sub>4</sub> + count.

**Statistical Methods**

Descriptive statistical analysis has been carried out in the present study. Results on continuous measurement are presented on mean SD (Min-Max) and results on categorical measurements are presented in number (%). Significance is assessed at 5% level of significance.

Chi-square/ Fisher's exact test has been used to find the significance of study parameters on categorical scale between two or more groups. 95% Confidence Interval has been computed to find the significant features. Confidence Interval with lower limit more than 50% is associated with statistical significance. The Statistical Software namely SPSS 16.0 Stata

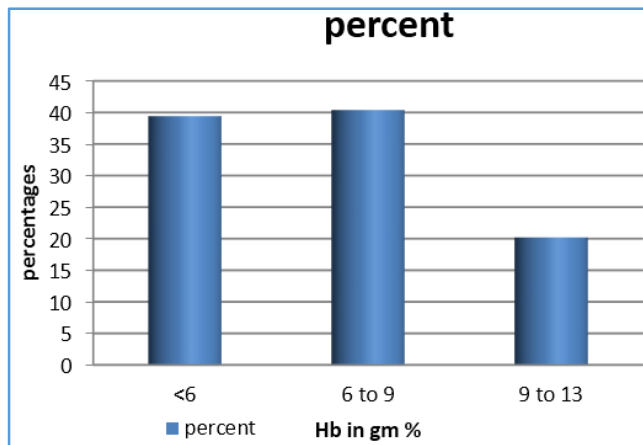
8.0 were used for the analysis of the data and Microsoft Word and excel have been used to generate graphs, tables etc.

**RESULTS**

Baseline haematological parameters.

HB	Frequency	Percent
< 6	41	39.42
6 to 9	42	40.38
9 to 13	21	20.19
<b>Total</b>	<b>104</b>	<b>100</b>

*Table 2. Distribution of Haemoglobin*

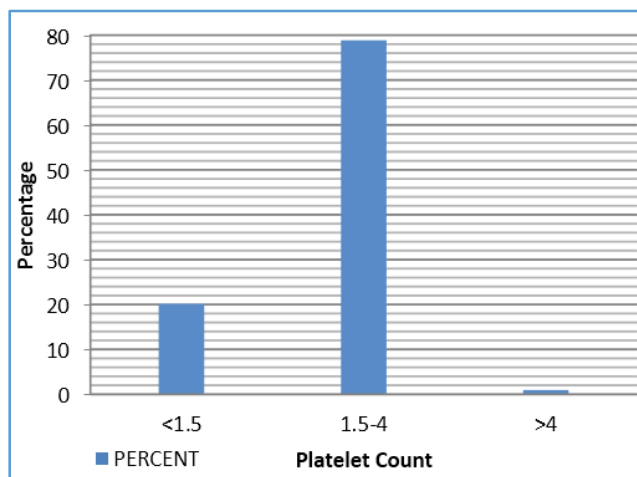


*Figure 1. Distribution of Haemoglobin*

Out of 104 patients in 42 patients, Hb% was between 6 and 9 gms%, 41 patients Hb% was < 6 gms% and 21 out of 104 patients were having Hb % 9 to 13 gm%.

Platelets	Frequency	Percent
<1.5	21	20.19
1.5-4	82	78.85
>4	1	0.96
<b>Total</b>	<b>104</b>	<b>100.00</b>

*Table 3. Distribution of Platelets*



*Figure 2. Distribution of Platelets*

The platelets count ranges from 0.3 to 4.2 lakhs/cmm<sup>3</sup> with prevalence of thrombocytopenia of about 20%.

Total Leucocyte Count	Frequency	Percent
<4000	46	44.23
4000-11000	41	39.42
>11000	17	16.35
<b>Total</b>	<b>104</b>	<b>100.00</b>

*Table 4. Distribution of Total Leucocyte Count*

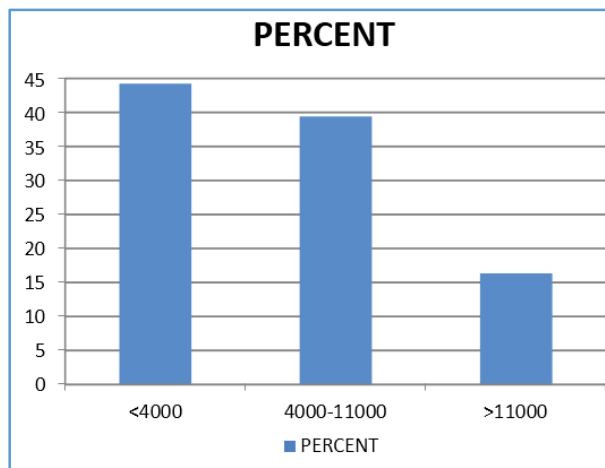


Figure 3. Distribution of Total Leucocyte Count

**Total Leucocyte Count**

The total leucocyte count ranged from 800 cells/mm<sup>3</sup> to 19,000 cells/mm<sup>3</sup> with out of 104 patients. The total leucocyte count between 4000 to 11,000 for 41 (39.42%) patients < 4000 for 46 (44.23%) patients and > 11,000 for 17 (16.35%) patients.

Bone Marrow Cellularity	AIDS 39		Non-AIDS 65		Total	P value
	Count	%	Count	%		
Normocellular	21	53.85	37	56.92	0.0011	
Hypocellular	12	30.77	4	6.15		
Hypercellular	6	15.38	24	36.92		
<b>Total</b>	<b>39</b>	<b>100.00</b>	<b>65</b>	<b>100.00</b>		

Table 5. Distribution of Bone Marrow Cellularity in HIV Patients

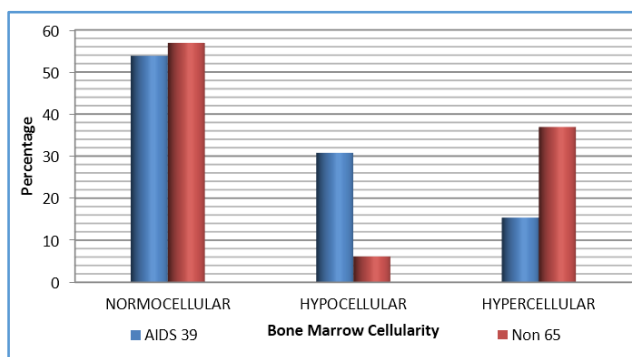


Figure 4. Distribution of Bone Marrow Cellularity in AIDS and Non-AIDS Patients

Amongst non-AIDS patients, normocellularity was the most common bone marrow picture seen in 37 out of 65 (56.92%) patients followed by hypercellularity and hypocellularity that were seen in 24 out of 65 (36.92%) and 4 out of 65 (6.15%) patients respectively. Even among AIDS patients, normocellularity was the most common bone marrow picture seen in 21 out of 39 (53.85%) patients followed by hypocellularity and hypercellularity that were seen in 12 out of 39 (30.77%) and 6 out of 39 (15.38%) patients.

Erythroid Dysplasia (n= 16)	Megaloblastic	Basophilic Stippling	Total
	12 (75%)	4 (25%)	16 (100%)
Granulocytic dysplasia (n= 6)	Cytoplasmic Vacuolation	Nuclear Dysmorphism	Total
	4(66.66)	2 (33.33)	6 (100%)
Megakaryocytic dysplasia (n= 2)	Hypolobulation		Total
	2 (100%)		2 (100%)

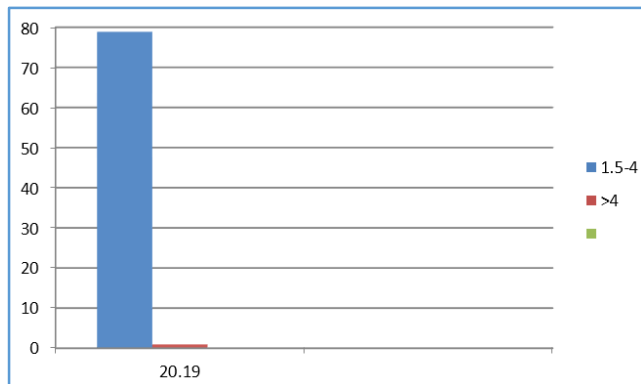
Table 6. Distribution of Type of Bone Marrow Dysplasia

Dysplastic changes in Erythroid series amongst a total of 16 patients were megaloblastoid changes in 75% (n= 12) and Basophilic stippling in 25% (n= 4) and almost all patients in this 87.5% (n= 14) were in Stage III and Stage IV. Amongst the patients with dysplastic changes in Granulocytic series (n= 6), Cytoplasmic vacuolation was seen in 66.6% (n= 4) and

nuclear dysmorphism in 33.3% (n= 2). All patients with granulocytic dysplasia were in Stage III and Stage IV. There were only two patients with dysplastic changes in Megakaryocytic series (n= 2), who had Hypolobulation and was in stage II.

Cytopenia	AIDS		Non-AIDS		Total	
	Count	%	Count	%	Count	%
Bicytopenia (B)	17	43.60	13	20.00	30	28.80
Pancytopenia(P)	6	15.40	4	6.20	10	9.60
1 cell line cytopenia (M)	116	41.00	46	73.60	54	61.50
<b>Total</b>	<b>39</b>	<b>100</b>	<b>65</b>	<b>100</b>	<b>104</b>	<b>100</b>

Table 7. Peripheral Haematological Abnormalities in Non-AIDS and AIDS Patients



**Figure 5. Peripheral Haematological Abnormalities in Non-AIDS and AIDS Patients**

Bicytopenia was seen in 30 (28.80%) out of 104 patients and it was seen in 13 (20%) of Non-AIDS and 17 (43.60%) of AIDS patients.

Pancytopenia was seen in 10 (9.60%) out of 104 patients and it was seen in 4 (6.20%) of non-AIDS and 6 (15.40%) of AIDS patients and only 1 cell line cytopenia seen in 64 (61.50%) out of 104 patients and it was seen in 48 (73.80%) of Non-AIDS and 16 (41%) of AIDS patients, both pancytopenia and bicytopenia are more common in AIDS than Non-AIDS patients.

Bone marrow examination was of diagnostic usefulness in patients with fever with anaemia, thrombocytopenia or leucopenia which was frequently seen in patients infected with HIV which would be of definite use in detecting infectious pathogens and HIV-associated malignancy.

(N= 104)	No. of Patients	%
AFB positive Granuloma	04	3.64
Histoplasma-capsulatum	01	.96
Parvovirus B-19	02	1.92
Hodgkin's lymphoma	01	.96

**Table 8. Specific findings in Bone Marrow related to Infectious Pathogen/ Malignancy**

4/104 patients, 3.84% patients showed epithelioid granulomas in bone marrow biopsies. All granuloma which were caseating epithelioid granuloma showed AFB positivity small clusters of histoplasma capsulatum along with lymphohistiocytic aggregates were seen in 1/104 patients (0.9%).

1/104 (0.9%) patients showed Reed-Sternberg (RS) cells in bone marrow and was of mixed cellularity.

2/104 of all patients and 2/90 of anaemic patients showed presence of giant pronormoblasts with clumped basophilic chromatin and clear cytoplasmic vacuoles made on bone marrow examination was suggestive of Parvovirus B19, later the diagnosis confirmed by in situ hybridization using sequence-specific DNA probes for Parvovirus B19.

**DISCUSSION**

Total patients included in the study were 104, out of which males were 66 and females were 38. Majority (80%) of the patients were in the age group of 21 - 40 years.<sup>8</sup> In the present study 62.5% (n= 65) were non-AIDS patients, while the rest 33.50% (n= 39) were AIDS patients.<sup>8</sup>

The most common indication for bone marrow aspiration was anaemia observed in 86.50% followed by leucopenia and thrombocytopenia, which were seen in 44.20% and 20.2% respectively.<sup>8,9</sup>

Anaemia, leukopenia and thrombocytopenia were more common in the patients with AIDS than in those with non-AIDS patients.

Normocytic normochromic anaemia was the commonest type of anaemia seen in 58 out of 90 patients (64.40%) followed by microcytic hypochromic anaemia seen in 26 out of 90 patients (28.90%) and macrocytic anaemia seen in 6 out of 90 patients (6.7%).<sup>8</sup>

Bone marrow was normocellular in 55.76% of the cases, hypercellular in 28.84 of the cases and hypocellular in 15.38% of the cases.<sup>8,10,11</sup>

The higher percentage of hypocellular bone marrow was observed in AIDS than in non-AIDS and was statistically significant.

Overall dysplasia was seen in 23.10% of the patients. Dysplasia was seen in 35.90% of AIDS patients as compared to only 15.4% of non-AIDS patients.

Erythroid dysplasia was the commonest dysplasia and was seen in 15.38% of all cases and 66.66% of all dysplasias.<sup>8</sup>

Lymphoid cells were decreased in 30.77% of non-AIDS and 56.41% of AIDS patients and this was statistically significant.

1 patient out of 104 patient's bone marrow showed Hodgkin's lymphoma.

**CONCLUSION**

- Peripheral and bone marrow abnormalities are common in HIV-related disease and have significant impact on clinical outcomes and quality of life (QOL).
- The variation in the prevalence of haematological abnormalities in different stages of disease are due to number of factors which includes CD4 count, clinical disease status, drug therapy and opportunistic infections.
- At present HIV infection is more prevalent.
  - a. In males as compared to females.
  - b. In the sexually active age group of 20 - 49 years.
- Most common presenting symptom are easy fatigability, weight loss and fever.
- Most common clinical signs are pallor and oral thrush.
- The incidences of Anaemia, Leukopenia and Thrombocytopenia are more in AIDS than in Non-AIDS.
- Normocytic normochromic anaemia is the most common peripheral haematological abnormality.
- Bone marrow was normocellular on majority of the patients.
- Hypocellular bone marrow is five times more common in AIDS than in Non-AIDS.
- Dysplasia is common in AIDS than in Non-AIDS.
- Erythroid dysplasia is the commonest type of dysplasia.
- Patients with AIDS have higher incidence of decreased bone marrow lymphoid cell count than Non-AIDS patients.

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