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To study the prevalence and severity of neutropenia in newly diagnosed HIV patients and their correlation with CD4 counts

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***Abstract*---**Background: The incidence as well as the severity of haematological complications increases as the HIV disease progresses. Neutropenia usually occurs as HIV disease progresses and is an independent risk factor for bacterial infection in advanced HIV disease. Method: this observational study was carried out in Department of Medicine, J.L.N. Medical College and hospital, Ajmer. 100 patients were selected for study and were examined and underwent routine investigations. Results: In this study the frequency and severity of leucopenia increases with declining CD4 counts and has got significant impact on clinical outcome.

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Introduction

The incidence as well as the severity of haematological complications increases as the HIV disease progresses. Neutropenia usually occurs as HIV disease progresses and is an independent risk factor for bacterial infection in advanced HIV disease. Progression of HIV infection is largely dependent on the interaction between viral factors and host factors. HIV primarily infects CD4 lymphocytes in the body. more than half of HIV infected patients have haematological complications, the incidence as well as the severity of these complications increases as the HIV disease progresses. The main haematological manifestation in HIV include anaemia, leucopenia, thrombocytopenia, coagulation disorders, haematological malignancies, etc. neutropenia usually occurs as HIV disease progresses and is an independent risk factor for bacterial infection in advanced HIV disease.

Leucopenia is common in HIV infected individuals.it generally correlates with severity of clinical syndrome like anemia.57 to 85 % of patients with fully developed AIDS are leucopenic.[1,2] leucopenia typically involves lymphocytes and granulocytes although monocytopenia has also been reported in AIDS patients.[3] A reduction in absolute number of CD4 T cells occurs as one of the earliest immunologic abnormalities of HIV infection. The reduction in CD4 lymphocytes is one of the most important prognostic indicators for the risk of developing opportunistic infections.

Number of morphologic abnormalities are seen in peripheral blood and bone marrow. neutrophils may show nuclear hyposegmentation with apparent left shift, presence of monolobed or pelger huet forms. increased size and prominent granulation are also seen in neutrophils. Large vacuolated monocytes and atypical lymphocytes are seen in patients who are lymphopenic.[3,4] Granulocytopenia is a common complication of several drugs used in AIDS related infections including trimethoprim-sulfamethoxazole, pentamidine, pyrimethamine –silfadiazine, flucytosine, ganciclovir.[5] Dose dependent neutropenia has been reported in 8-16 % of patients during long term therapy.[6] neutropenia in AIDS patients is associated with an increased incidence of infection with bacterial and fungal pathogens. Most common bacterial infections are staphylococcal infections, fungal infections are Cryptococcus and candida species. They are also associated with pulmonary aspergillosis, pyomyositis, malignant external otitis, neutropenic enterocolitis and pseudomonas keratitis....abnormalities in neutrophil function have also been described in AIDS patients including impairment of chemotaxis and phagocytosis, reduced expression of adhesion molecules on neutrophils, production of toxic oxygen free radicals.[7]

Mechanism of neutropenia in AIDS patients is multifactorial it could be due to secondary infection, infiltrative malignancies in bone marrow , direct or indirect effect of HIV infection, drug complications and nutritional deficiencies .possible autoimmune mechanism for destruction of peripheral blood granulocytes is suggested by presence of antineutrophil antibodies in 67 % of patients with AIDS.[8] Neutropenia may also be related to abnormal regulation of cytokines with

depressed levels of granulocyte colony stimulating factor (G-CSF).[6] There is some evidence that bone marrow stromal cells may be infected with HIV thus altering bone marrow microenvironment.[6] Alexandra et al. studied hematological manifestation in 1729 HIV infected patients, which demonstrated that neutropenia is associated with more advanced HIV disease and it also showed that neutropenia can be prevented by antiretroviral therapy in patients with normal neutrophil count.[9]

Aims and objectives

1. To study the prevalence and severity of neutropenia in HIV patients.
2. To assess the correlation of severity of neutropenia with their CD-4 counts.

Materials and Methods

This study was carried out in departments of medicine, JLN Medical College and hospital ajmer.in this observational study 100 patients were selected who attended the medical opd and admitted in various medical wards. and underwent routine and specific investigations.

Inclusion Criteria

All HIV positive (ELISA three kit positive) patients.

Exclusion criteria

1. Patient who was having active bleeding
2. Patients who were having following infections; (a). Mycobacterial infections (b). fungal infections (c). B19 parvovirus infections
3. Patients who were on following drugs; Zidovudine, dapsone, trimethoprim/ sulfamethoxazole, pyremethamine, 5-flucytosine, Ganciclovir, Interferon-alpha, Trimetrexate, Foscarnet
4. Patients on other drugs which may lead to bone marrow suppression;
   a. cytotoxic drugs used in cancer chemotherapy, alkylating agents, antimetabolites, antimitotics
   b. chloramphenicol
   c. insecticides
   d. antiprotozoals: quinacrine and chloroquine, mepacrine
   e. non-steroidal anti-inflammatory drugs (including phenylbutazone, sulindac, indomethacin, ibuprofen, aspirin)
   f. anticonvulsants-hydantoins, phenacemide, felbamate, carbamazepine.
   g. Heavy metals- gold, arsenic, bismuth, mercury
   h. some antibiotics (sulfonamides), anti-thyroid drugs (methimazole, methylthiouracil, propylthiouracil), antidiabetic drugs (chlorpropamide, tolbutamide), carbonic anhydrase inhibitors (acetazolamide and methazolamide)
   i. antihistamines (chlorpheniramine, cimetidine)
   j. D-penicillamine
   k. Estrogens (in pregnancy and in high doses in animals)

General physical examination and systemic examination with special focus on signs of hematological involvement like pallor, icterus, any evidence of bleeding manifestation, hepatomegaly, splenomegaly, lymphadenopathy, and opportunistic infection was noted. Routine investigations: hemoglobin (by sahli acid hematin method), TLC (by using neubar method), DLC (PBF with Leishman stain), ESR (by westergreen method), serum bilirubin (Diazo method), Serum creatinine (Jaffe method).

Special investigations:

- CD4 count by flow cytometery,
- ELISA test by ELISA three kit test – Micro ELISA, Tridot, Quick Check
- Chest X-ray PA view
- Clinical, Radiological and bacteriological evidence of pulmonary tuberculosis, histoplasmosis, pneumocystis jiroveci pneumonia, cerebral toxoplasmosis

For this study the following diagnostic parameters were taken:
Neutropenia- absolute neutrophil count < 1000/cumm

Observations

These 100 patients were divided into following three groups based on symptoms and CD4 counts.
Group 1: CDC stage A1, A2 which includes asymptomatic HIV patients and CD4 counts more than 200 per cu mm.
Group 2: CDC stage B1, B2 which includes patients with symptomatic HIV infections but without AIDS defining conditions, and CD4 counts above 200 per cumm.
Group 3: CDC stage A3, B3, C1, C2, C3 which includes patients with immunological or clinical AIDS.
The group 1 includes total 41 patients (Male 22, Female 19)
Group 2 includes total 16 patients (male 5, female 11)
Group 3 includes total 43 patients (male 25, female 18)
In the present study for the frequency of Hb distribution the observation are as follows

Table 1: Age and Sex Distribution

Age group (years)	Group 1 (CD 4>200 cells / cumm) CDC stage A1, A2 M = Male; F = Female (n)			Group 2 (CD 4>200 cells / cumm) CDC stage B1, B2 M = Male; F = Female (n)			Group 3 (CD 4 < 200 cells / cumm) CDC stage A3, B3, C M = Male; F = Female (n)			Total M = Male; F = Female (n)		
	M (22)	F (19)	Total (41)	M (5)	F (11)	Total (16)	M (25)	F (18)	Total (43)	M (52)	F (48)	T (100)

16-30	6	13	19	1	7	8	3	8	11	10	28	38
31-40	11	4	15	2	4	6	13	7	20	26	15	41
41-50	5	2	7	2	-	2	5	2	7	12	4	16
>50	-	-	-	-	-	-	4	1	5	4	1	5

M = Male; F = Female

Age and sex distribution

In this study we have screened 100 patients divided into 3 groups: Group I have 41 patients, Group 11 having 16 patients and Group III having 43 patients.

Group I

- Out of 41 patients screened (Male = 22, Female = 19), the Male: Female ratio was 1.15: 1.
- The Age ranges from 16 years to 50 years, and the mean age was 32.8 years.
- Most of the Male were in the Age group 31 to 40 years comprising 50% of the total male patients.
- Most female patients were in the age group of 16-30 years comprising 68.4%.
- Overall, in this group the age band 16-30 years comprises maximum number of patients 46.20% (19 out of 41) followed by age group 31-40 comprising 21.053 % (15 out of 41) followed by age group (41-50 years) comprising 17.07% (7 out of 41 patients).

Group II

- This group comprises 16 patients (Male- 5, Female -11). with =M: F ratio 1:2.2
- Age ranges 16-50 years
- Mean age is 31.5 years.
- Most males belong to the age group 31 to 40 years and 41 to 50 years {(40% in each group) (2 out of 5 patients in each group)}.
- Most females belong to the age band 16-30 years 63.6% (7 out of 11) followed by age group 31-40 years comprises of 36.30% (4 out of 11).
- In this group the age group 16-30 years comprises 50% of cases (8 out of 16 patients) followed by the age group (31 - 40 years) 37.5% (6 out of 16 patients), followed by the age group 41 – 50 years comprising 12.5%, (2 out of 16 patients).

Group III

- Total 43 patient were screened in this group (Male 25, Female = 18).
- Age ranges from 23-56 years, Mean age was 38.11 years.
- Most male patients in this group were in the age band 31 - 40 years - 52% (13 out of 25 patients)
- Most Female patients in this group were in the age band 16-30 years -44% (8 out of 18 patients)

Overall in this group:

- Maximum patients are in the age band (31 - 40 years) comprsing 46.5% (20 out of 43 patients).
- This is followed by the age band 16-30 years comprising 25.58% (11 out of 43 patients), followed by the age band 41 - 50 years comprising 16.27% patients (7 out of 43 patients) and
- The least number of patients were in the age band > 50 years comprising 11.6% (5 out of 43 patients).

For total 100 patients

- Among all the 100 patients screened in the three categories, Male 52, Female = 48. Most males were in the age band 31 – 40 years (50% of the male population) (26 out of 52 patients)
- Female patients in the age band (16-30 years) comprises 58.33% of the total population (28 out of 48 patients)
- While the age band > 50 years comprises least number of patients – 7.6% of male population (4 out of 52 patient) and 2% of female population (1 out of 48 patients)
- If sex distribution is ignored than the maximum no. of patients were in age band 31-40 years (41%) and the least no. of patients are in age band 50 years comprising 5% of total patient population.

Table 2: Frequency of TLC Distribution

TLC / cumm	Group 1 (CD 4>200 cells / cumm) CDC stage A1, A2 M = Male; F = Female (n)			Group 2 (CD 4>200 cells / cumm) CDC stage B1, B2 M = Male; F = Female (n)			Group 3 (CD 4 < 200 cells / cumm) CDC stage A3, B3, C M = Male; F = Female (n)			Total M = Male; F = Female (n)		
	M (22)	F (19)	Total (41)	M (5)	F (11)	Total (16)	M (25)	F (18)	Total (43)	M (52)	F (48)	T (100)
>11000	1	1	2	-	-	-	1	1	2	2	2	4
4000-11000	21	18	39	3	10	13	15	10	25	39	38	77
<4000	-	-	-	2	1	3	9	7	16	11	8	19

M = Male; F = Female

Frequency of TLC distribution

The observations for the frequency of the TLC distribution in various groups are as follows:

Group I

- For the 41-patient screened in this group (M-22, F=19), The TLC ranges from 4000-12600/Cu.mm with the mean TLC is 7548/Cu.mm and Std. dev. is 2064 cells/cu. mm.
- Most of the Male 95.4% (21 out of 22 patients) have their TLC between 4000-11000 cells/cu.mm.
- Only 4.54% (1 out of 22 patient) have TLC > 11000.
- Most females 94.73% (18 out of 19 patients) have their TLC between 4000-11000 cells/cu.mm.
- Here also only 5.26% of the female patients (1 out of 19 patients) have TLC>11000 cells/cu.mm.
- No patients in either group had leucopenia.
- P value is >.05 hance no correlation exists between CD4 & TLC.

Group II

- For the 16 patients screened TLC count ranges from 1800 – 9350 cells/cu.mm.
- The mean TLC was 5953.126 cells/cu.mm. with S.D. 2177 cells/cu.mm.
- Here most of the male - 60 % (3 out of 5 patients) have their TLC count between 4000-11000 cells/cu.mm.
- 40% of patient (2 out of 5) have TLC <4000/cells/cu.mm.
- 90.9% of the female (10 out of 11 patients) have TLC in the range of 4000-11000 cells/cu.mm.
- No female has TLC count > 11000 cells/cu.mm.
- 9.09% female (1 out of 11) have TLC <4000.
- Overall, 18.7% (3 out of 16 patients) have TLC < 4000 cells/cu.mm and 81.25% cases (13 out of 16 patients) have TLC between 4000 - 11000 cells/cu.mm.
- P value is < .05 hence the result is significant, and correlation exist between CD4 & TLC for group II.

Group III

- For the 43 patients screened (M-25, F-18) the TLC range from 1000 - 13100 cells/cu.mm., The mean TLC was 5378 cells/cu.mm. and 1 S.D.=2807 cells/cu.mm.
- Here also most of the Males - 60% (15 out of 25 patients) have their TLC between 4000-11000 cells/cu.mm.
- This is followed by the TLC band < 4000 cells/cu.mm. which comprises 36% of the male patients (9 out of 25 patients).
- Only 4% of the males (1 out if 25 patients) have TLC > 11000 cells/cu.mm.
- Most females 55.5% (10 out of 18 patients) have TLC count between 4000 11000 cells/cu.mm. followed by TLC band < 4000 cells/cu.mm. which comprises 38.88% (7 out of 18 patients)
- Only 5.55% of Female (1 out of 18 patient) had TLC > 11000 cells/cu.mm.

In this group

- 58.14% patients (25 out of 43 patients) have TLC count between 4000-11000 cells/cu.mm.
- 37.2% (16 out of 43 patients) have TLC <4000 cells/cu.mm.
- 4.65% (3 out of 43 patients) have TLC > 11000 cells/cu.mm.
- P value is < .05 hence the result significant CD4 the correlation exists between CD4 & TLC group III.

Overall assessment of TLC revealed that

- 3.84% of the Male (2 out 52 patients) and 4.16% of the Females (2 out of 48 patients) have TLC > 11000 cells/cu.mm.
- 75% of the Male (38 out of 52 patients) and 79.16% of the females (39 out of 48 patients) have TLC between 4000 -11000 cells/cu.mm.
- Only 21.154% of the male (11 out of 52 patients) and 16.66% of the female (8 out of 48 patients) have TLC <4000 cells/cu.mm.
- Out of 19 leucopenic patients 16 were in group III comprising maximum number of leucopenic patients.

So, the complete picture is:

- TLC band > 11000 cells/cu.mm comprises 4% cases
- TLC band 4000-11000 cells/cu.mm. comprises 77% cases
- TLC band <4000 cells/cu.mm. comprises 19% cases

Table 3: Frequency of ANC Distribution

ANC / cumm	Group 1 (CD 4>200 cells / cumm) CDC stage A1, A2 M = Male; F = Female (n)			Group 2 (CD 4>200 cells / cumm) CDC stage B1, B2 M = Male; F = Female (n)			Group 3 (CD 4 < 200 cells / cumm) CDC stage A3, B3, C M = Male; F = Female (n)			Total M = Male; F = Female (n)		
	M (22)	F (19)	Total (41)	M (5)	F (11)	Total (16)	M (25)	F (18)	Total (43)	M (52)	F (48)	T (100)
>7500	1	1	2	-	-	-	1	1	2	2	2	4
1000-7500	21	18	39	5	11	16	21	15	36	47	44	91
<1000	-	-	-	-	-	-	3	2	5	3	2	5

M = Male; F = Female

(C) Frequency of absolute neutrophil count distribution
GROUP I

In this group for the 41-patient screened (M-22, F=19) the ANC ranges from 2350 - 8650 cells/cu.mm with mean ANC = 4766 cells/cu.mm with S.D. 1536 cells/cu.mm

- The most of the males-95.56% (21 out of 22 patient) have the ANC in the range of 1000-7500 cells/cu.mm. Only 4.54% of the patients (1 out of 22 patient) have ANC >7500 cells/cu.mm.
- Most females - 94.7 % (18 out of 19 females) have the ANC in the range 1000 7500 cells/cu.mm. Only 5.26% females (1 out of 19 Patients) have the ANC > 7500 cells/cu.mm.
- No patient in either group have ANC <1000 cells/cu.mm.
- Overall, 4.878% of the patients (2 out of 41 patients) have ANC > 7500 cells/cu.mm and 95.1% of the patients (39 out of 41 patients) have ANC in the range of 1000-7500 cells/cu.mm.
- P value is >.05 hence the result is not significant & correlation dosent exist between CD4 & ANC group I.

GROUP II

For the 16 patients screened (M=5, F=11) ANC ranges from 1100 - 6084 cells/cu.mm, mean ANC was 3464 cells/cu.mm with S.D. 1586 cells/cu.mm.

- 100% of the male patients (5 out of 5 patients) have the ANC between 1000-7500 cells/cu.mm
- Similarly, 100% to female (11 out of 11 patients) have ANC between 1000-7500 cells/cu.mm.
- No patient in either group have ANC < 1000 or ANC >7500 cells/cu.mm.
- P value is <05 hence the result is significant & correlation exist between CD4 & ANC group II.

GROUP III

For the 43-patient screened (M-25, F-18) the ANC ranges from 588 12833 cells/cu.mm, the means ANC is 3375 cells/cu.mm and S.D. is 2360 cells/cu.mm.

- In the male patients 4% (1 out of 25 patient) have ANC > 7500 cells/cu.mm, 84% of the patient (21 out of 25 patient) have ANC between 1000-7500 cells/cu.mm and 12% (3 out of 25 patient) have ANC<1000 cells/cu.mm.
- Most of the Female 83.33% (15 out of 18 patient) have ANC between 1000-7500 cells/cu.mm. followed by 5.55% female (1 out of 18 patient) have ANC > 7500 cells/cu.mm and followed by that 11.11% cases (2 out of 18 female patients) have ANC < 1000 cells/cu.mm.

Overall, in this group

- 83.72% (36 out of 43 patients) have their ANC between 1000 -7500 cells/cu.mm and
- 11.6% (5out of 43) have ANC range < 1000 cells/cu.mm and
- 4.65% (2 out 43) have ANC >7500 cells/cu.mm
- P value is <.05 hence the result is significant & correlation exist between CD4 & ANC group III.

The complete scenario of frequency of ANC distribution in all the groups combinedly –

- 91% patients have ANC in the normal range 1000 – 7500 cells/cu.mm.
- 4% patients having high ANC (7500 cells/cu.mm)
- 5% patients having low ANC (< 1000 cells/cu.mm)

Discussion

Age And Sex Distribution

Table 4: Sex distribution of the cases in various studies in relation to our study

Age & Sex	Imtiaz et al	Attilli et al	Our study
M:F Ratio	4:1	3.9:1	1.15:1
Mean Age (years)	33.80	34	34.8

The result of the data analysis obtained from our study consisting of 100 patients (M = 52, F = 48) shows M: F ratio 1.15: 1 which is significantly lower than reported by Imtiaz et al (4: 1) and Attilli et at (3.9:1). Cause of the difference in the M: F ratio is probably due to difference in the prevalence of HIV in different states. Coming to the age distribution the age ranges in our study from 16 years to 56 years with mean age of 34.8 years which is similar to mean age of presentation reported by Imtiaz et al -33.8 years and Attilli et al – 34 years.

41% of the patients fall in the age group 31-40 years and followed by the age group (16 upto 30 years) in which there are 38% cases, so these two-group comprising 79% of the total patient population are the most productive and sexually active age group. This finding is accordance to that of Imtiaz et al where 74% of the patient fall in the age group 21-40 years. In the present study in comparison to males, Females are younger, 58.3% females are in the age group 16-30 years as compared to only 19% male in this age group, maximum number of males are in the age group 31-40 years which contain 50% of the males. The older age group contain the least percentage of patients (5% of total patient population).

Total Leucocyte count

Table 5: Leucopenia in various studies in relation to our study

Study	Imtiaz et al n= 100	Attili et al N = 470	Bharat et al N= 61	Akinbami et al n = 205	Our study n = 100
Leucopenia	40 %	13-44 %	29.3 %	26.8 %	19 %

Table 6: Leucocytosis in various studies in relation to our study

Study	Imtiaz et al	Ours
Leucocytosis	Nil	4 %

The TLC in our study varies in the different groups:

- Group I - 4000-12600 cells/cu.mm, mean TLC-7405 cells/cu.mm.
- Group II - 1800-9350 cells/cu.mm, mean TLC-7548 cells/cu.mm.
- Group III 1000 - 13100 cells/cu.mm, mean TLC – 5953 cells/cu.mm.

Leucopenia (TLC < 4000 cells/cu.mm.) was found in 19% total patients in our study this is significantly lower than the finding reported by Imtiaz et al (40%), Attilli et al (13-44%), Bharat et al (29.3%) and Akinbami et al (26.8%) probably because of in our study we have excluded the patient who are already on ART or having Tubercular infection or on Chemotherapeutic agents mentioned in the exclusion criteria. The other studies have not used the exclusion criteria.

We have observed that as the stages progresses the prevalence of leucopenia increases, percentages ge of the patients having Leucopenia in various groups are as follows:

- Group 1 – Nil
- Group II - 18.75
- Group III-37.2% (36% males and 38% females)

This finding correlates with that of Attili et al where leucopenia varies from 13-40% according to the disease state. Hence, they appear to be strong correlation between CD4 & TLC (especially in group II, III) (P value <0.05 significant).

Leucocytosis

It is present in about 4% of total patients. The prevalence of leucocytosis in various groups are as follows:

- Group I -4.8%
- Group II – Nil
- Group III -4.65%

Hence there seems to be no correlation between leucocytosis and immunological status.

Absolute neutrophil count

Table 7: Neutropenia in various studies in relation to our study

Study	Attili et al	Moreno gracia et al [4,5]	Ours
Neutropenia	13-40 %	8-50 %	5 %

The ANC ranges in the various groups are as follows:

- Group 1: 2350-8600 cells/cu.mm. (mean ANC 4766 cells/cu.mm)
- Group II: 1100-6084 cells/cu.mm. (mean ANC 3375 cells/cu.mm)
- Group III: 588-12833 cells/cu.mm (mean ANC 3761 cells/cu.mm)

The prevalence of neutropenia varies widely according to the disease stage. In our studies we have found 5% of the patients with ANC < 1000 this is significantly lower as reported by Attili et al (13 -40%), Moreno Gracia et al (8-50%) because these studies have majority of patients on ART or other Chemotherapeutic agents which are included in our exclusion criteria. We observed that all the neutropenic patients fall in Group III also mean TLC is lowest in Group III which gives the clue that as the disease progresses the prevalence and severity of neutropenia increases. (P <0.05) hence a strong correlation exists between ANC & CD4

Conclusion

- In this study 100 patient were screened for the common hematological finding, neutropenia.
- The frequency and severity of neutropenia increases with declining CD4 counts and has significant impact on clinical outcome.

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