ORIGINAL RESEARCH

A study of haematological abnormalities with correlation to CD4 count as immunological marker in HIV infected individuals prior and after highly active antiretroviral therapy

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ABSTRACT

Background:HIV is a global pandemic with a prevalence of 0.3% as in 2015 according to NACO.

Materials & Methods: Blood sample of 300 HIV positive patients on HAART was collected from ART centre, KIMS, Bangalore. CD4 count, complete hemogram and peripheral smear was studied in comparison to their HAART naïve status over a period of 18 months.

Results: A total of 300 HIV positive patients were evaluated, pre and post HAART in which anaemia was the most common haematological abnormality. Leucopenia, lymphopenia, neutropenia & thrombocytopenia were other abnormalities observed, all of which correlated with CD4 count & disease progression. Post HAART, there is significant improvement in all these abnormalities proving the efficacy of HAART.

Conclusion: Haematological abnormalities such as anaemia, leucopenia, leucocytosis, neutropenia, neutrophilia, lymphopenia, lymphocytosis and thrombocytopenia are common in HIV infected individuals.Anaemia is the most common haematological abnormality and most frequent type is normocytic normochromic anaemia. Post HAART macrocytosis is common in occurrence due to the effect of zidovudine.HAART is an effective therapy as it improves haematological parameters and haematological parameters can be used as an alternative to CD4 count in resource poor settings.

Key words: HIV, AIDS, Haematological abnormalities, HAART, CD4 count

INTRODUCTION

HIV is a global pandemic with a prevalence of 0.3% as in 2015 according to NACO.[1] AIDS is a disease caused by retrovirus, human immunodeficiency virus characterized by profound immunosuppression, haematological abnormalities, opportunistic infections and neoplasms.[1]Haematological abnormalities like anaemia, leucopenia, leucocytosis and thrombocytopenia are important clinical manifestations. These constitute the second most common cause of morbidity and mortality in HIV patients. NACP (National AIDS Control Programme) has been implemented by govt. of India which aims at reducing new infections and providing support, care & treatment to the people living with HIV/AIDS.[2]Anaemia is reported to be the most common haematological abnormality. HIV infected individuals with anaemia are at risk of progression to AIDS and increased mortality, while treating the anaemia can decrease the risk.[3] Neutropenia is seen in advanced stage of the disease and has several causes. Lymphopenia and thrombocytopenia are also a common manifestation and correlates with severity of the disease.[4] CD4 count is essential for assessment of immune status in HIV infected person. Decreased absolute CD4 count attributes in the severity of the disease. HAART improves the CD4 counts in HIV-positive patients and ultimately corrects all the

haematological abnormalities.[5] The objective of the study to analyze the haematological abnormalities in correlation with CD4 count in HIV positive subjects, comparative analysis of immunological and haematological parameters of HAART naive and those on HAART, to study the age and sex distribution in subjects receiving HAART and the differences in immunological and haematological parameters.

MATERIALS & METHODS

The present study consisted of 300 HIV positive individuals registered in ART centre, KIMS of both genders. All gave their written consent to participate in the study. Data such as name, age, gender etc. was recorded. Complete haemogram was studied with automated analyzer Mindray BC-3000 using 5ml of EDTA blood sample collected via venepuncture with necessary aseptic precautions. Haematological parameters were counter checked with a Leishman's stained peripheral smear for confirmation of the diagnosis. CD4 count was done using standardised automated flow cytometer FACS Calibur. in our pre HAART study group there are only 2 categories CD4 < 200/µL & CD4 > 200/µL whereas 3 groups in post HAART i.e. CD4 < 200/µL, 200-500/µL &> 500/µL. Data thus obtained were subjected to statistical analysis. P value < 0.05 was considered significant.

RESULTS

Table: I Age distribution of patients

Age in years	No. of patients	%
20-30	32	10.7
31-40	107	35.7
41-50	104	34.7
51-60	37	12.3
61-70	17	5.7
>70	3	1.0
Total	300	100.0

Table: I shows that the mean ± SD age of the patients was 42.13±10.33 years.

Table II: Comparison of pre and post-HAART variables

Variables	Pre HAART	Post HAART	Difference	t value	P value
CD4	204.83±88.87	561.94±266.70	-357.113	-23.083	<0.001**
WBC	5.66±2.16	6.31±1.88	-0.640	-4.285	<0.001**
Lymph	1.59±0.84	2.14±0.76	-0.548	-9.348	<0.001**
MID	0.33±0.27	0.41±0.30	-0.088	-3.856	<0.001**
Gran	3.73±1.61	3.81±1.49	-0.088	-0.758	0.449
lymph%	28.14±9.46	34.17±9.17	-6.029	-8.842	<0.001**
MID%	5.75±3.88	6.34±1.48	-0.606	-2.518	0.012*
Gran%	65.66±10.47	58.70±11.23	6.956	8.163	<0.001**
Hb	11.24±2.20	12.62±2.12	-1.386	-9.301	<0.001**
RBC	4.16±0.88	3.42±0.69	0.743	12.471	<0.001**
HCT	33.69±6.57	31.22±5.19	2.468	5.939	<0.001**
MCV	81.18±13.05	89.56±11.16	-8.382	-9.607	<0.001**
MCH	27.10±4.92	38.27±5.54	-11.174	-29.225	<0.001**
MCHC	-	41.26±1.97	-	-	-
PLT	2.10±0.88	232.23±76.07	-230.127	-52.524	<0.001**

Student t test (Paired) Table II shows that there was a significant difference in the pre and post HAART values of CD4 count, total WBC count, absolute lymphocyte count, haemoglobin, RBC count, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin and platelet count with p value of <0.001.

Table: III Comparison of anaemia in pre HAART cases with CD4 count

Hb		CD4 count <200			CD4 count 200-500			Total
M	F	M	F	Total	M	F	Total	
>13	≥12	24(27.9%)	15(23.4%)	39(26%)	30(44.1%)	20(24.4%)	50(33.3%)	89(29.7%)
12.9-10	11.9-10	38(44.2%)	23(35.9%)	61(40.7%)	30(44.1%)	39(47.6%)	69(46%)	130(43.3%)
9.9-8	9.9-8	17(19.8%)	17(26.6%)	34(22.7%)	6(8.8%)	15(18.3%)	21(14%)	55(18.3%)
<8	<8	7(8.1%)	9(14.1%)	16(10.7%)	2(2.9%)	8(9.8%)	10(6.7%)	26(8.7%)
Total		**86(100%)**	**64(100%)**	**150(100%)**	**68(100%)**	**82(100%)**	**150(100%)**	**300(100%)**

*Total cases of anaemia=211(70.33%), Fisher Exact test There were 37% & 33.33% cases of anaemia in pre HAART patients in the category of CD4 count <200/µL &>200/µL respectively The number of anaemia cases were maximum in pre HAART patients in the category of CD4 count <200/µL i.e. 74%.

Table: IV Comparison of anaemia in post HAART cases with CD4 count

Hb		CD4 count <200			CD4 count 200-500			CD4 >500		
M	**F**	**M**	**F**	**Total**	**M**	**F**	**Total**	**M**	**F**	**Total**
>13	>12	10 (71.4 %)	9 (90 %)	19 (79.2 %)	33 (58.9 %)	32 (66.7 %)	64 (76.2 %)	50 (56.8%)	114 (66.3 %)	198 (66%)
12.9-10	11.9 -10	3 (21.4 %)	1 (10 %)	4 (16.7 %)	20 (35.7 %)	9 (18.8 %)	18 (21.4 %)	26 (29.5%)	44 (25.6 %)	77 (25.7 %)
9.9-8	9.9-8	1 (7.1 %)	0 (0%)	1 (4.2%)	2 (3.6%)	4 (8.3%)	0 (0%)	9 (10.2%)	9 (5.2%)	16 (5.3%)
<8	<8	0 (0%)	0 (0%)	0 (0%)	1 (1.8%)	3 (6.3%)	2 (2.4%)	3 (3.4%)	5 (2.9%)	9(3%)
Total		**14 (100 %)**	**10 (100 %)**	**24 (100 %)**	**56 (100 %)**	**48 (100 %)**	**104 (100 %)**	**84 (100%)**	**88 (100%)**	**172 (100 %)**

*total cases of anaemia =102(34%), Fisher Exact test There were 1.66% & 32.33% cases of anaemia in post HAART patients & in the category of CD4 count <200/µL &>200/µL respectively. Hence total improvement in hemoglobin was observed in 36.33% of cases. The number of anaemia cases were least in the post HAART patients in the category of CD4 count of >500/µL i.e. 33.7%. Number of cases of anaemia (Hb <10gm/dl) decreased from 20.8% to 8.6% & 33.33% to 20.6% in post & pre HAART cases in the category of CD4 <200/µL& CD4 >200/µL respectively. This suggests severity of anaemia is decreasing with the increasing CD4 count favouring a positive correlation.

Table: V Correction of haematological parameters after HAART in the present study

No of cases of (out of 300)	Pre HAART	Post HAART	P value
Anaemia	70%	34%	<0.001**
Leucocytosis	21%	9%	0.003**
Leucopenia	2.3%	0.66%	0.049*
Neutropenia	10%	6.33%	0.056
Neutrophilia	4.33%	4.66%	0.417
Lymphopenia	24.33%	3%	<0.001**
Lymphocytosis	4%	11.66%	<0.001**
Thrombocytopenia	29.6%	11%	<0.001**
Thrombocytosis	1.33%	1.33%	1.00

Paired Proportion test The difference between number of cases of anaemia, leucocytosis, lymphopenia, lymphocytosis, thrombocytopenia in pre & post HAART is statistically significant with p value <0.001 whereas it is insignificant for leucopenia, neutropenia, neutrophilia, thrombocytosis.

DISCUSSION

Haematological manifestations are documented as one of the most common cause of morbidity and mortality in HIV patients.[6] Most commonly encountered haematological abnormalities in HIV-infected individuals are anaemia, granulocyte disorders, thrombocytopenia, lymphomas, coagulopathies and vascular malignancies like Kaposi sarcoma. Although in the majority of cases, haematologic abnormalities are detected in middle or advanced stages of HIV infection, anaemia and thrombocytopenia have been reported to occur in early stages of HIV infection.[7,8]The origin of haematological disorders in HIV individuals is attributed to multiple factors like severe nutritional stress in advanced stages of HIV infection, dysfunctional bone marrow due to direct effect of virus, suppression of marrow by invading opportunistic infections or neoplasm, chronic disease associated changes and toxic side effects of antiretroviral drugs (or other medications used to combat the complications of HIV disease.[9,10] The objective of the study to analyze the haematological abnormalities in correlation with CD4 count in HIV positive subjects, comparative analysis of immunological and haematological parameters of HAART naive and those on HAART, to study the age and sex distribution in subjects receiving HAART and the differences in immunological and haematological parameters.We found that the mean ± SD age of the patients was 42.13±10.33 years. Rahman et al[11]studied bone marrow aspiration findings in HIV-positive patients and their correlation with CD4 count. 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.We found that there was a significant difference in the pre and post HAART values of CD4 count, total WBC count, absolute lymphocyte count, haemogloblin, RBC count, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin and platelet count. There were 37% & 33.33% cases of anaemia in pre HAART patients in the category of CD4 count <200/µL &>200/µL respectively The number of anaemia cases were maximum in pre HAART patients in the category of CD4 count <200/µL i.e. 74%. Attili et al[12] studied the spectrum of hematological manifestations and evaluated the relationship between various hematological manifestations and CD4 cell counts. A total of 470 HIV-infected individuals were followed for 830 person years of observation (PYO). Rate of hematological episodes was 1047 episodes per 1000 PYO. CD4 counts were significantly lower in individuals with severe anemia and neutropenia compared to those without. However, no relation could be established between thrombocytopenia and CD4 counts. CD4 levels were significantly lower in those with anemia/neutropenia harboring any particular disease compared to those who had the same disease without anemia/ neutropenia.

We observed that there were 1.66% & 32.33% cases of anaemia in post-HAART patients & in the category of CD4 count <200/µL &>200/µL respectively. Hence total improvement in hemoglobin was observed in 36.33% of cases. The number of anaemia cases was least in the post-HAART patients in the category of CD4 count of >500/µL i.e. 33.7%. The number of cases of anaemia (Hb <10gm/dl) decreased from 20.8% to 8.6% & 33.33% to 20.6% in post &pre-HAART cases in the category of CD4 <200/µL& CD4 >200/µL respectively. This suggests severity of anaemia is decreasing with the increasing CD4 count favouring a positive correlation.We found that the difference between the number of cases of anaemia, leucocytosis, lymphopenia, lymphocytosis, and thrombocytopenia in pre &post-HAART is statistically significant whereas it is insignificant for leucopenia, neutropenia, neutrophilia, thrombocytosis. Pande et al[13] found that the majority of the patients had normocytic-normochromic anemia (63%), in tune with the available data. In most of the cases bone marrow was hypercellular (63.04%), although in a significant proportion it was found to be hypocellular (19.57%). Erythropoiesis was suppressed in 36.96% of patients. Dysplastic changes involving isolated cell lines ranged from 13.04% to 45.65%, dysmegakaryopoiesis being the most common, followed by dyserythropoiesis. Marrow plasmacytosis was detected in 23.91% of patients. No statistically significant correlation was detected in between immunological status (CD4 count) and marrow cellularity, myelodysplastic changes or marrow plasmacytosis. In a fair number of cases bone marrow examination aided in the diagnosis of opportunistic infections.

CONCLUSION

Authors found that haematological abnormalities such as anaemia, leucopenia, leucocytosis, neutropenia, neutrophilia, lymphopenia, lymphocytosis and thrombocytopenia are common in HIV infected individuals.Anaemia is the most common haematological abnormality and most frequent type is normocytic normochromic anaemia. Post HAART macrocytosis is common in occurrence due to the effect of zidovudine.HAART is an effective therapy as it improves haematological parameters and haematological parameters can be used as an alternative to CD4 count in resource poor settings.

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