HAEMATOLOGICAL PROFILE IN HIV PATIENTS AND ITS CORRELATION WITH WHO CLINICAL STAGING

Hisanath Beegum[1], Prasantha Kumar Thankappan[2], Mohanan Peruntharamolel Pappuni[3]

[1]Resident, Department of Medicine, Government Medical College, Kottayam, Kerala, India.
[2]Additional Professor, Department of Medicine, Government Medical College, Kottayam, Kerala, India.
[3]Professor, Department of Medicine, Government Medical College, Kottayam, Kerala, India.

ABSTRACT

BACKGROUND

The aim of the study is to study the spectrum of haematological manifestations and CD4 counts and evaluate the relationship between haematological manifestations and CD4 counts in relation to WHO clinical staging in a hospital-based study of HIV infected adults in and around Kottayam.

MATERIALS AND METHODS

Clinical and haematological profile of patients attending medicine department and antiretroviral therapy clinic, Kottayam, were recorded. The relationship between WHO clinical staging and CD4 counts and various haematological manifestations was analysed.

RESULTS

A total of 120 HIV infected individuals, in which, 71 were males and 49 were females. Candidiasis was most common opportunistic infection followed by tuberculosis. Anaemia and elevated ESR was most common haematological abnormality. Anaemia (Pearson correlation -0.827), ESR (Pearson correlation +0.78), total lymphocyte count (Pearson correlation -0.807), CD4 counts (Pearson correlation -0.944) were correlating with WHO clinical staging and falling tendency of these parameters were statistically significant, but total count and platelet count had no correlation with WHO clinical staging or falling tendency were not correlating with progression of disease.

CONCLUSION

Anaemia, elevated ESR was the most common haematological manifestation. CD4 count, total lymphocyte count can be used to assess the progression of disease in HIV patients. CD4 count is best indicator of disease progression.

KEYWORDS

Haematological Profile, CD4 T-Cell Count, Correlation with Clinical Staging.

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BACKGROUND

Haematopoietic disorders are common throughout the course of HIV infection. It can be due to direct effect of HIV or due to secondary infection or neoplasms. These haematological abnormalities will increase with disease progression.

Anaemia is the most common haematological abnormality in HIV infection, prevalence range from 1.3 to 95%, normocytic normochromic is most predominant type followed by microcytic.

Thrombocytopenia is the second most frequent haematological abnormality found in HIV patients ranging from 3 to 40%. Autoimmune mediated destruction have been postulated as the reason for this. During the course of infection, neutropenia maybe seen in approximately half of patients. Severe neutropenia found in advanced disease.

More severe haematological abnormalities are see in advanced disease. We can roughly assess the disease progression by using this.

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Corresponding Author:
Dr. Prasantha Kumar Thankappan,
Additional Professor, Department of Medicine,
Government Medical College, Kottayam – 686008, Kerala.
E-mail: drprasantha@gmail.com
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MATERIALS AND METHODS

Study Design- The study is hospital-based cross-sectional study over a period of 12 months from June 2013 to June 2015 conducted in the Internal Medicine and Obstetrics and Gynaecology Departments of Government Medical College, Kottayam.

Study Subjects- All newly-detected patients with HIV infection aged 15 years or above proved by immunological assays, admitted to medical, obstetric and gynaecological wards or attending the ART Unit of Government Medical College, Kottayam, during the period June 2013 to June 2015 were considered as study subjects.

Inclusion Criteria

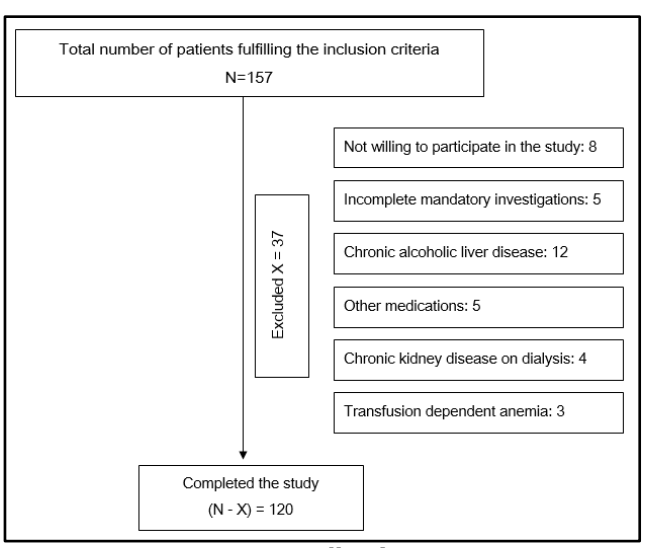
All cases of serologically proven new HIV infection aged 15 years above were considered for the study.

Exclusion Criteria

1. All patients with unrelated documented chronic diseases that are likely to alter the haematological profile like chronic renal or hepatic diseases, malignancies and haematological diseases.
2. HIV infected patients on drugs that are likely to alter the haematological profile including antiretroviral therapy and those on indigenous medications.
3. HIV infected patients not willing to participate in the study.

Study Procedure- Patients fulfilling the inclusion and exclusion criteria were considered for the study. Detailed history was taken to unveil the risk factors and the symptoms. All were subjected to clinical examination and were investigated. Basic haematological and biochemical investigations were done in all patients. In addition to the mandatory investigations, special investigations were done in patients to detect associated disease as and when the clinical settings warranted. Stage wise data were compiled and the means were compared using one-way ANOVA test. The correlation between assessed using Pearson coefficient analyses. The validity of the CDC staging system was assessed with the help of one-sample T test. The entire data haematological values were divided into quartiles using statistical softwares and the sensitivity, specificity, positive and negative predicative values were calculated for quartile limits with each haematological parameter in an attempt to stage the disease with parameters other than CD4 lymphocyte count. The observations were analysed in comparison with available literature and conclusions were made.

Statistical Analysis- The data collected was analysed with appropriate statistical methods with the help of Microsoft Excel and SPSS software under the guidance of statistician.



Data Collection

Sex Profile- Among the 120 patients included in the study, 71 were males and 49 were females. Sex ratio was detected to be 1.45:1 with male preponderance.

Age Distribution- Of the 120 patients studied, 87 belonged to the age group 25-49 years. Seventeen patients were below and sixteen patients were above this age group.

Risk Factor Profile- Among the study population, sexual contact was the only risk factor in 90%. Sexual contact was only risk factor in 48 of the total 49 female study subjects. The contact was heterosexual in all female patients. Among male patients, 6 gave history of homosexual contact in addition on the heterosexual contacts and one male patient had homosexual contacts only, while 43 had heterosexual contacts as the only risk factor. Fifty nine male patients and twenty female patients had sexual contact with more than one heterosexual partner. Among the male patients with multiple heterosexual partners, 52 had contact with commercial sex workers, while rest had contact with others (neighbours, friends, co-workers and relatives). Among the 5 male patients with a single heterosexual partner, 4 had contact with commercial sex worker only and one had contact only with his spouse. Among the female patients with multiple partners, 7 were commercial sex workers, while the rest had contact with others (neighbours, friends, co-workers and relatives). Rest 28 females had only a single sexual partner. Two male patients never had sexual contacts, but were injectable drug abusers. Nine male patients had both injectable drug usage and high-risk sexual (heterosexual) behaviour. One male never had a sexual contact and used to have recurrent blood leans fusion. None of the patients with high-risk sex behaviour used barrier contraceptives regularly nor used post exposure prophylaxis. Ninety eight of the study subjects were married and spouse was seropositive in 81 cases. 60% of subjects had children, among which 45.83% had children seropositive for HIV infection.

Sex	Risk		n	%	%
Females (49)	Sexual contact	Heterosexual	48	97.96	97.96
		Homosexual	0	0	
		Combined	0	0	
	Percutaneous contact	Injectable drug	0	0	2.04
		Accidental	1	2.04	
		Transfusion	0	0	
	Both		0	0	
	Transplacental		0	0	
Males (71)	Sexual contact	Heterosexual	53	74.64	84.50
		Homosexual	1	1.41	
		Combined	6	8.45	
	Percutaneous contact	Injectable Drug	2	2.82	2.82
		Accidental	0	0	
		Transfusions	0	0	
	Both		9	12.68	12.68
	Transplacental		0	0	0

Table 1. Risk Profile of Study Subjects

WHO Clinical Staging- Of the total 120 subjects, 31 comprising of 18 female and 13 males belonged to WHO clinical stage 1. Twenty one, 11 females and 10 males had WHO stage 2 disease, while 33, 9 females and 24 males were found to suffer from WHO stage 3 disease. A 35

patients, 24 males and 11 females suffered from WHO stage 4 infection.

Haematological Profile- The haematological parameter evaluated included haemoglobin level, total leucocyte count, total lymphocyte count, platelet count, mean corpuscular volume, reticulocyte count and the CD4 count. The most common haematological abnormalities were elevated erythrocyte sedimentation rate (N=98), anaemia (N=96), positive direct Coombs test (N=44), reticulocytopenia (N=32) and thrombocytopenia (N=17). Other haematological abnormalities included leucopenia (N=9), leucocytosis (N=4) and a case of reticulocytosis. The mean haemoglobin levels (gm/dL) were 12.81, 10.99, 9.18 and 7.17 in WHO clinical stages 1, 2, 3 and 4, respectively. The total mean leucocyte counts (cells/mm^3) were 7961.29, 7890.48, 7551.52 and 4995.14 and mean platelet counts (lakhs/mm^3) were 2.81, 2.78, 2.74 and 1.47 in stages 1, 2, 3 and 4, respectively. The mean erythrocyte sedimentation rates (mm/hr.) were 20.03, 36.43, 59.09 and 92.63 in clinical stages 1, 2, 3 and 4, respectively. Mean total lymphocyte counts (cells/mm^3) were 3783.13, 3251.76, 2497.18 and 1397.11, while mean CD4 counts (cells/mm^3) were 606.61, 403.57, 284 and 103.86 in stages 1, 2, 3 and 4, respectively. The mean 'mean corpuscular volumes' (fl) in stages 1, 2, 3 and 4 were 88.6, 87.90, 89.64 and 87.44. A total of 44 patients (36.67%) had a positive Direct Coombs Test (DCT) of which 27 belonged to clinical stage 4 and 15 belonged to stage 3. One patient each from clinical stage 1 and 2 also showed a positive Coombs reactivity. Peripheral smear showed evidence of haemolysis in one patient and the patient had positive Coombs reactivity. Six patients had plasmacytosis in peripheral smear, all had clinical stage 4 disease.

Haemoglobin	Stage 1	Stage 2	Stage 3	Stage 4
Anaemia	10	18	33	35
Anaemia-Haemoglobin	<13 g/dL (males >15 years) <12 g/dL (non-pregnant females >15 years)			

Table 2. Haematological Abnormalities in the Study Group

ESR	Stage 1	Stage 2	Stage 3	Stage 4
Elevated	13	18	33	34
Normal ESR	Males- 0 -15 mm/hour			
	Females- 0-20 mm/hour			
Reticulocyte Count	**Stage 1**	**Stage 2**	**Stage 3**	**Stage 4**
Reticulocytopenia	11	5	6	10
Reticulocytosis	0	0	0	1
Normal reticulocyte count	Males- 0.8-2.3%			
	Females- 0.8-2.0%			

Table 3. Retic Count and ESR

Platelet Count	Stage 1	Stage 2	Stage 3	Stage 4
Thrombocytopenia	0	0	1	16
Normal platelet count	1.5-4.5 lakhs/mm^3			

Table 4. Comparison of Thrombocytopenia

Direct Coombs Test	Stage 1	Stage 2	Stage 3	Stage 4	Total
Positive	1	1	15	27	**44**
Negative	30	20	18	8	**76**
Positive %	3.33%	5.00%	45.45%	71.14%	**36.67%**

Table 5. Comparison of Direct Coombs Test

CD4 Count	Stage 1	Stage 2	Stage 3	Stage 4
>500	30	1	0	0
200-499	1	20	32	1
<200	0	0	1	34
CDC staging system	Stage 1- CD4 count >500 cells/mm^3 Stage 2- CD4 count 200-499 cells/mm^3 Stage 3- CD4 count <200 cells/mm^3			

Table 6. CD4 Count

	Mean	SD	Median
WHO Stage 1			
Haemoglobin	12.81	1.46	13.20
Total leucocyte count	7691.29	2022.04	7300
Platelet count	2.81	0.75	2.78
Haemoglobin	12.81	1.46	13.20
Total leucocyte count	7691.29	2022.04	7300
Mean corpuscular volume	88.6	2.81	88
Reticulocyte count	0.97	0.43	0.8
CD4 count	606.61	102.49	563
WHO Stage 2			
Haemoglobin	10.99	1.49	11.20
Total leucocyte count	7890.48	2132.58	7300
Platelet count	2.78	0.58	2.75
Erythrocyte sedimentation rate	36.43	14.37	35
Total lymphocyte count	3251.76	806.94	3050
Mean corpuscular volume	87.90	3.08	88
Reticulocyte count	1.19	0.48	1.10
CD4 count	403.57	47.46	393
WHO Stage 3			
Haemoglobin	9.18	1.18	8.90
Total leucocyte count	7551.52	2218.12	7200
Platelet count	2.74	0.66	2.75
Erythrocyte sedimentation rate	59.09	16.42	57
Total lymphocyte count	2497.18	473.10	2356
Mean corpuscular volume	89.64	4.01	89
Reticulocyte count	1.18	0.47	1.10
CD4 count	284	37.73	288
WHO Stage 4			
Haemoglobin	7.17	1.77	7.90
Total leucocyte count	4995.14	1952.57	4900
Platelet count	1.47	0.59	1.50
Erythrocyte sedimentation rate	92.63	34.79	89
Total lymphocyte count	1397.11	454.06	1364
Mean corpuscular volume	87.44	3.79	88
Reticulocyte count	1.05	0.64	0.80
CD4 count	103.86	53.10	92

Table 7. Statistical Analysis of Haematological Parameters

Haemoglobin and WHO Clinical Stage- As the clinical stage of the disease advanced, the patient's haemoglobin tended to drop serially. The fall in haemoglobin was found to be statistically significant (ANOVA; P.000) and the haemoglobin level correlated well with the clinical stage of

the disease (Pearson correlation; -0.827). A haemoglobin level above the third quartile (11.8 g/dL) has a positive predictive value and sensitivity of 77.42% with a negative predictive value and specificity of 92.13% in predicting a stage 1 disease, while haemoglobin level below the first quartile (8.2 g/dL) has a positive predictive value of 89.29% and a sensitivity of 96.47% in predicting a stage 4 disease. A haemoglobin level between the first and the third quartiles has a positive predictive value of 72.13% and a sensitivity of 81.48% with a negative predicative value of 83.05% and specificity of 74.24% in predicting a stage 2/3 disease.

Total Leucocyte Count and WHO Clinical Stage- As the clinical stage of the disease advanced, the patients total leucocyte count tended to drop serially, but the same was found not be statistically significant between clinical stages 1, 2 and 3 (ANOVA; P0.84). The fall in stage 4 was noticed to be statistically significant (ANOVA: P.00). There existed no significant correlation between total leucocyte count and clinical stage in stages 1, 2 and 3, but the total leucocyte count in stage 4 correlated with the clinical stage of the disease (Pearson correlation -0.528). Leucopenia (<4000 cell/mm^3) had a positive predictive value of 87.5% and a sensitivity of 20% with a negative predictive value of 75% and specificity of 98.82% in predicting a stage 4 disease in the present study.

Platelet Count and WHO Clinical Stage- As the clinical stage of the disease advanced, the patient's platelet count tended to drop serially. The fall in platelet count as found not to be statistically significant between clinical stages 1, 2 and 3 (ANOVA; P0.922), but the fall in stage 4 was found to be statistically significant (ANOVA; P0.00). There existed no significant correlation between the platelet count and clinical stage in stages 1, 2 and 3, but the platelet count in stage 4 correlated well with the clinical stage of the disease (Pearson correlation -0.720). Thrombocytopenia (<1.5 lakh cells/mm^3) had a positive predictive value of 94.12% and a sensitivity of 45.71% with a negative predictive value of 81.55% and specificity of 98.82% in predicting a stage 4 disease according to this study.

Erythrocyte Sedimentation Rate and WHO Clinical Stage- As the clinical stage of the disease advanced, the patient's erythrocyte sedimentation rate tended to increase serially. The rise in erythrocyte sedimentation rate as found to be statistically significant (ANOVA P0.00) and correlated well with the clinical stage of the disease (Pearson correlation +0.778). An erythrocyte sedimentation rate above the third quartile (74.25 mm/hr.) has a predictive value of 90% and specificity of 95.29% in predicting a stage 4 disease, while a erythrocyte sedimentation rate below the first quartile (26.25 mm/hr.) has a positive predictive value of 80% and a sensitivity of 77.42% with a negative predictive value of 93.25% and specificity of 93.26% in predicting a stage 1 disease. An erythrocyte sedimentation rate between the first and the third quartiles has a positive predictive value of 75% and a sensitivity of 83.33% with a negative predictive value of 85% and specificity of 77.27% in predicting a stage 2/3 disease.

Total Lymphocyte Count and WHO Clinical Stage- As the clinical stage of the disease advanced, the patient's total lymphocyte count tended to decline serially. The fall in total lymphocyte count was found to be statistically significant (ANOVA; P0.000) and correlated well with the clinical stage of the disease (Pearson correlation -0.807). A total lymphocyte count above the third quartile (3412.72 cells/mm^3) has a positive predictive value of 76.67% and sensitivity of 74.2% with a negative predictive value of 91.11% and specificity of 92.13% in predicting a stage 1 disease, while a total lymphocyte count below the first quartile (1748.25 cell/mm^3) has a positive predictive value of 94.44% and specificity of 100% in predicting a stage 4 disease. A total lymphocyte count between the first and the third quartiles has a positive predictive value of 78.33% and a sensitivity of 87.04% with a negative predictive value of 88.33% and specificity of 80.30% in predicting a stage 2/3 disease.

Mean Corpuscular Volume and WHO Clinical Stage- There was neither statistically significant trend in the mean corpuscular volume with advancing stage of the disease (ANOVA; P0.07) nor existed a definite correlation between mean corpuscular volume and WHO clinical staging of the disease.

Reticulocyte Count and WHO Clinical Stage- There was either statistically significant trend in the reticulocyte count with advancing stage of the disease (ANOVA; P 0.35) nor existed a definite correlation between reticulocyte count and WHO clinical staging of the disease.

CD4 Count and WHO Clinical Stage- As the clinical stage of the disease advanced, the patient's CD4 count tended to decline serially. The fall in CD4 count was found to be statistically significant (ANOVA; P0.000) and correlated well with the clinical stage of the disease (Pearson correlation -0.944). The validity of Centers for Disease Control (CDC) laboratory staging system using the CD4 count was assessed in our study population. In WHO stage 1 disease, the CD4 count at a test value of 500 had a mean difference of +106.61 (one sample t test; P0.000) and in WHO stage 4 disease at a CD4 test value of 200. There was a mean difference of -96.143 (one sample t test; P0.000). For WHO stags 2 and 3 together at a CD4 test value of 200, there was a mean difference of 130.50 (one sample t test; P0.000) and a t test value 500. There was a mean difference of -169.50 (one sample t test; P0.000). A CD4 lymphocyte count above 500 cells/mm^3 has a positive predictive value and sensitivity of 96.77% with a negative predictive value and specificity of 98.88% in predicting a stage 1 disease, while a CD4 lymphocyte count below 200 cells/mm^3 has a positive predictive value and sensitivity of 97.14% with a negative predictive value and specificity of 98.82% in identifying a stage 4 disease. CD4 lymphocyte counts between 200 and

500 cells/mm^3 had a positive predictive value and sensitivity of 96.30% with a negative predictive value and specificity of 96.97% in diagnosing a stage 2/3 disease.

Among the various haematological parameters studied, CD4 lymphocyte count had the best correlation with the WHO staging of the disease and is also the best haematological marker to predict the disease stage.

Direct Coombs Test and WHO Clinical Stage- As the clinical stage of the disease advanced, the direct Coombs test tended to become positive. Positive direct Coombs test correlated well with the advancing clinical stage of the disease (Pearson correlation +.621).

WHO Clinical Stage		1	2	3	4	Total
DCT	Positive	1	1	15	27	**44**
	Negative	30	20	18	8	**76**
	Positive %	3.33%	5.00%	45.45%	71.14%	**36.67%**

Table 8. Comparison of DCT in WHO Clinical Stage

Direct Coombs Test (DCT) in predicting the stage of the disease.

Stage / DCT	Advanced (Stages ¾)	Early (Stages ½)
Positive	42	2
Negative	26	50

Table 9. DCT in Disease Progression

- Sensitivity- 6176%.
- Specificity- 96.15%.
- Positive predictive value- 95.45%.
- Negative predictive value- 65.79%.

A positive direct Coombs test has 61.76% sensitivity and 96.15% specificity for identifying and advanced stage (WHO clinical stages 3/4). HIV infection with a positive predictive value of 95.45% and a negative predictive value of 65.79%.

DISCUSSION

The emergence and pandemic spread of human immunodeficiency virus constitute the greatest challenge to public health in modern time. The first case of human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) in India was detected in 1986, and since then, the spread of HIV/AIDS across the nation has been relentless. The clinical spectrum of HIV infection in India is different from that in the rest of the world. There is also great variability within India as well. Haematological manifestations are the second commonest cause of morbidity and one of the common causes of mortality in HIV patients.[1,2,3] Though there are a few published data on the haematological manifestations of HIV infection from the Indian subcontinent, there is no such data from the state of Kerala. Since Kerala differs from rest of the Indian states in many aspects, the data regarding the clinical and haematological manifestations from these states are not likely to be applicable to Keralites.

Age and Sex- HIV infection was detected in all age groups above 15 years, but 72.5% of the cases belonged to the 25-49 years age group. 13.3% of the infected belonged to the group 50 years or more, while 14.2% were young adults as defined by UNAIDS as 15-24 years age. The maximal distribution in the age group 25-49 years is in accordance to most of the available published data. According to the NACO[2] India HIV Estimates; 88.7% of infected in the country and 87.99% of infected in Kerala belonged to the age group 15-49 years.[1] Except in young adult, a male preponderance was identified with a total male-to-female ratio 1.45:1, the ratio being lower than the NACO estimate of 1.56:1 as well as the UNAIDS regional estimate of 1.61:1[1] maybe because of the better accessibility to the healthcare system for females and higher female-to-male sex ratio in the state. Many other published data from the country has documented a much higher male-to-female case ratio.

Haematological Profile- The most common haematological abnormalities identified in the study population were elevated erythrocyte sedimentation rate (81.67%), anaemia (80%), positive direct Coombs test (36.67%), reticulocytopenia (26.67%) and thrombocytopenia (14.17%). In different study settings, the prevalence of anaemia in persons with AIDS has been estimated at 63% to 95%[3] making it more common than thrombocytopenia or leucopenia in patients with AIDS. The most frequent cause of anaemia in HIV infected patients is anaemia of chronic disease. According to literature, thrombocytopenia is believed to be present in as many as 40% of HIV-infected persons and in approximately 10% of the patients, it may be the first sign of AIDS. The prevalence of anaemia in our study population remains similar to that in literature, but the prevalence of thrombocytopenia is noticeably low. There are no published data regarding the incidence of reticulocytopenia and elevated erythrocyte sedimentation rate in patients with HIV/AIDS.

Haemoglobin- A progressive fall in the haemoglobin level was noticed as stage of the disease advanced. The mean haemoglobin levels were 12.81, 10.99, 9.18 and 7.17 gm/dL. A large epidemiological study on anaemia in HIV-infected patients published by the Division of HIV/AIDS, Center for Disease Control and Prevention including more than 32,000 HIV infected from January 1990 to August 1996 indicates that 28% of men and 31% of women with asymptomatic HIV infection were anaemic (haemoglobin <14 and <12 g/dL, respectively). The figure rose to 87% and 77% respectively for patients with clinically manifest AIDS. Other studies have also shown that a low haematocrit or haemoglobin level is associated with progression of HIV disease or a decrease in CD4+ counts. The Kigali staging system has included a haematocrit less than 0.38 as a criterion for identifying stage 3/4 disease.[4,5] Various studies have also identified that those without anaemia regardless of the CD4 cell count at the time of initial diagnosis. Haemoglobin has also been demonstrated to be strongly associated with progression of HIV infection both in

untreated and treated and it is suggested that haemoglobin of <10.6 g/dL may provide guidance on when to recommend antiretroviral therapy according to Wanjari et al.[4,6,7] Difference in the mean haemoglobin levels between stages was found to be statistically significant levels correlated well with the clinical stage of the disease (Pearson Correlation -0.827 at 0.0 significance). A haemoglobin level of less than 8.2 gm/dL strongly predicted a WHO stage 4 disease according to our study. According to a study by R Omoregie et al, haemoglobin below 12 g/dL significantly predicted CD4 cell counts below 200 cells/mm^3. The lower cutoff in our study is compared to the western population and greater socioeconomic constraints in our population of HIV infected.

Total Leucocyte Count- The total leucocyte count tended to fall with advancing stage of the disease, but a statistically significant drop was noticed only in stage 4 HIV infections. A falling tread of neutrophils and lymphocytes was demonstrated in studies by Attili SVS et[8] al and Kasthuri A.S. et al.[4] Another study in 1988 showed that 13% of patients with asymptomatic HIV infection had neutropenia, while the incidence rose to 44% in clinical AIDS. The drop in total leucocyte count correlated with the stage of the disease only in the most advanced disease stage in the present study. Published data[8,4,9] has shown that the incidence of neutropenia was 0.8% in HIV-positive patients with CD4 count >700 cells/mm^3; it rose to 13.4% in those with CD4 count <249 cells/mm^3.

Platelet Count- As in the case of total leucocyte count, platelet count dropped significantly and correlated well with WHO clinical staging only in stage 4 disease. Thrombocytopenia is well known to occur all throughout the course of HIV infection irrespective of the disease stage. However, in this study, it is noticed that thrombocytopenia is more common in stage 4 disease. Studies by Dominguez and co-workers in 41 HIV-infected thrombocytopenic patients found that platelet survival was lower in those with CD4 counts above 200 cells/mm^3 than in those with counts below this level implying that platelet destruction is more important in patients with high CD4 counts and decreased platelet production is more important in those with lower CD4 counts. This variance from the western literature in our tertiary care setting. The study by Kasthuri A.S. et al[4,9] has also demonstrated a higher incidence of thrombocytopenia in advanced stages of the disease. Although, 8% of patients with HIV-associated thrombocytopenia will have haemorrhagic event according to literature, none of our patients had bleeding manifestations. The mean platelet count in stage 4 disease was calculated to be 1.47 lakhs/mm^3. The coefficient of correlation of -0.720 was noticed between platelet count and WHO stage 4 disease. An almost similar and nonsignificant fall in platelet count was noticed in a study by Attili SVS et al.[8]

Erythrocyte Sedimentation Rate- The study by Alan R. Lifson et al has demonstrated clearly that the erythrocyte sedimentation rate is significantly raised in advanced disease and an ESR of >65 is now a marker of Kigali stage 3/4 disease. According to the same study and few other studies, erythrocyte sedimentation rate strongly predicted survival in HIV infected. In accordance to these large published studies, the erythrocyte sedimentation rate tended to increase with advancing stage in our study also. The mean erythrocyte sedimentation rates (mm/hr.) were 20.03, 36.43, 59.09 and 92.63 in clinical stages 1, 2, 3 and 4 respectively in this study and the difference between the means was found to be significant (ANOVA one-way analysis, P 0.000). The rise in erythrocyte sedimentation rates correlated with the advancing disease stage (Pearson correlation +0.778 at 0.01 significance). In this study, an erythrocyte sedimentation rate of above 74.25 had significantly predicted a WHO stage 4 disease with a positive predictive value of 86.67%. According to the study by Alan R. Lifson et al an elevated erythrocyte sedimentation rate of >65 mm/hr. significantly predicted an advanced disease and prognosis. Till now, there is no published data on the relationship between erythrocyte sedimentation rate and HIV infection from the Indian subcontinent.

Total Lymphocyte Count- Total lymphocyte count has been studied in various trials within and outside the country as a marker of disease severity since long years and is considered as the second best available marker to CD4 count in predicting the disease stage and the degree of immunosuppression. It has been suggested as an alternative to CD4 count in staging the disease in resource limited developing countries. The laboratory axis of WHO staging system is based on this parameter. The correlation between total lymphocyte count and CD4 count is a subject of interest for a few decades by now. This study shows a steady and progressive decline in total lymphocyte count with advancing disease stage. This is in according with available published literature.[5,10] The mean total lymphocyte counts (cell/mm^3) were 3783.13, 3251.76, 2497.18 and 1397.11 in stages 1, 2, and 3, respectively. According to a study by Wilhan and Budiono, the mean total lymphocyte count in HIV/AIDS patients with CD4 below 200 cell/mm^3 was 1013.24 ± 96.06. In our study, the fall in total lymphocyte count was found to be statistically significant (ANOVA one-way analysis; P0.000) and correlated well with the clinical stage of the disease (Pearson Correlation -0.807 at 0.01 significance). A total lymphocyte count of <1748.25 cell/mm^3 had an 85.71% sensitivity and 100% specificity to identify a WHO stage 4 disease. According to a study by Spacek et al from United States of America, a total lymphocyte count of <1200 cell/mm^3 had a sensitivity of 70.7% and specificity was 81.7% for identifying a case with CD4 less than 200 cells/mm^3. The higher sensitivity and specificity in our study is probably because of the higher cutoff that has been selected in our study.

CD4 Count- CD4 lymphocyte count is now considered as the gold standard marker for the severity of immunosuppression in HIV infection. It is the laboratory maker for disease staging according to the staging system

proposed by the Centers for Disease Control and Prevention (CDC). In our study with advancing clinical stage of the disease, the CD4 count dropped serially with an excellent statistical correlation. The mean CD4 counts (cells/mm^3) were 606.61, 403.57, 284 and 103.86 in stages 1, 2, 3 and 4, respectively. The difference in the mean CD4 counts between stages was found to be extremely significant (ANOVA one-way analysis P0.000). According to a study by Edathodu et al from Saudi Arabia, the mean CD4+ T-lymphocyte counts were 457, 337, 188 and 86 cells/mm^3 at the respective stages and the difference between the mean CD4+ T-lymphocyte count in patients at stage IV and at each of the other stages was significant; $p < 0.0001$. The fall in CD4 count and the disease stage correlated well with each other (Pearson correlation -0.944 at 0.01 significance) in our study. Excellent correlation between CD4 count and the WHO clinical staging has been demonstrated by a multitude of studies by now.

Reticulocyte Count and Mean Corpuscular Volume- Both reticulocyte count and mean corpuscular volume failed to demonstrate any significant trend with advancing disease stage (ANOVA one-way analysis; 0.35 and 0.07 for reticulocyte count and mean corpuscular volume, respectively) and neither correlated with the WHO staging system.

Direct Coombs Test- Review of published literature suggests that direct antiglobulin (Coombs) test is positive in 37% of HIV-infected persons, but clinically significant haemolysis is a rare.[4] This indicates that positive Coombs test in HIV infection may simply be a reflection of polyclonal hypergammaglobulinemia, which is common in HIV infection. In accordance to the available published data, 36.67% of the study population had positive direct Coombs test in our study and only one case had evidence of haemolysis in the peripheral blood film. The incidence of positive direct Coombs test increased with advancing stage of the disease. A positive Coombs test correlated with the stage of the disease (Pearson correlation +0.621 at 0.01 significance).

Comparing CDC Staging System with WHO Staging System- The three tiered Centers for Disease Control system based on the CD4 lymphocyte count is considered to be the gold standard in staging HIV infection and guiding treatment in a developing country in all HIV infected due to technical and financial constraints. The WHO clinical staging system is considered to be an excellent alternative to the CDC system in such a resource limited setting. Evaluating the WHO staging as an alternative to CDC system in our study population, the WHO clinical stage 1 had positive predictive value and sensitivity of 96.78% with a negative predictive value and specificity of 98.88% for identifying patients with Centers for Disease Control stage 1 disease. The positive predictive value and sensitivity of the WHO clinical stage 4 were 97.14% using CDC stage 3 disease as the positive standard with a negative predictive value and specificity of 98.82%. The WHO clinical stages 2 and 3 combined had positive predictive value and sensitivity of 96.30% for identifying patients with CDC stage 2 disease with a negative predictive value and specificity of 96.97%. Most of the available published studies comparing WHO clinical staging with CDC staging has demonstrated an excellent concordance between the two systems.

Alternatives for CD4 Lymphocyte Count in Predicting the Stage of the Disease- The WHO clinical staging system maybe a good alternative in developing countries to the CD4. Lymphocyte count-based HIV staging system used in the developed world. A similar result is available from most published literature worldwide. The haemoglobin level, total lymphocyte count, erythrocyte sedimentation rate and positive Coombs test correlates well with the CD4 count. Strong association between CD4 counts and the above-mentioned haematological parameters have been demonstrated previously in literature.[10,11] Haemoglobin had the best correlation with CD4 count followed by erythrocyte sedimentation rate and total lymphocyte count ($r = +0.879$, -0.772, +0.771, respectively). Most studies in literature have demonstrated a better correlation for total lymphocyte count than for haemoglobin and erythrocyte sedimentation rate. Some studies have shown that adding haemoglobin to total lymphocyte count increased sensitivity of identifying advanced disease thereby reducing the risk of false-negative results.

CONCLUSION

1. Elevated erythrocyte sedimentation rate and anaemia are the most common haematological abnormalities in HIV infection.
2. Positive direct Coombs test is a common haematological abnormality in advanced stages of HIV infection.
3. CD4 lymphocyte count is the haematological parameter with the best correlation with WHO clinical staging of HIV infection.
4. Haemoglobin level, erythrocyte sedimentation rate and total lymphocyte count correlated well with the WHO clinical staging of HIV infection.
5. Thrombocytopenia is found to be significantly common in WHO clinical stage 4 HIV infection.
6. HIV infection is found to be more common in males than females.
7. HIV infection is more common in the sexually and economically productive age group.
8. Heterosexual contact is the single most common risk factor identified among the HIV infected.
9. Injectable drug use and male sex with male are less common high-risk behaviours among HIV infected.
10. The most common complaints at presentation are fatigue and fever followed by weight loss.

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