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*Original Research Article*

# Haematological Parameters in PLHIV with Utility of Absolute Lymphocyte Count (ALC) As Surrogate Marker for CD4 Count: A Study from India

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**Abstract**

**Context:** Since it's first report in 1982, HIV has emerged as a major pandemic. Haematological abnormalities are among the most common clinicopathological manifestations of HIV infection including impaired haematopoiesis, immune mediated cytopenias and coagulopathies.

**Aims:** To study haematological parameters in PLHIV and to correlate them with CD4 count. To correlate Absolute Lymphocyte Count (ALC) with CD4 count to evaluate the utility of ALC as a surrogate marker for CD4 count.

**Settings and Design:** Prospective observational study

**Methods and Material:** After obtaining informed consent, 225 PLHIV were clinically evaluated and subjected to CBC, PBS, ALC, ESR and CD4 count.

**Statistical analysis used:** Data was expressed as percentage and mean±standard deviation. Statistical tests used were chi-square test, Fisher's exact test and Mann-Whitney U test and Kolmogorov-smirnov analysis. Also, Pearson's rank order correlation and ROC curve was used.

**Results:** The important haematological findings noted were anaemia (n=123,54.6%), leucopenia(n=34,15.2%), lymphocytopenia (n=128,56.9%), thrombocytopenia (n=10,4.4%) and raised ESR (n=162,72%). CD4 count was reduced in 156 cases (69.3%). Haemoglobin, MCV, MCH and ALC ($p<0.0001$) showed significant positive correlation with CD4 count. ALC ≤ 1400cells/µl indicated CD4<200cells/µl.

**Conclusions:** The most frequent haematological finding is lymphocytopenia followed by anaemia. Haemoglobin, MCV, MCH and ALC showed rise with increase in CD4 count. ALC ≤ 1400cells/µl can act as surrogate marker for CD4<200cells/µl in resource poor settings.

**Keywords:** Absolute Lymphocyte Count; CD4 Count; ESR; Haematological Parameters; PLHIV.

## Introduction

WHO-UNAIDS estimated that currently 36.7 million people live with HIV infection world over (end 2015). According to WHO, an estimated 35 million people have died from AIDS related causes including 1.1 million in 2015 [1]. Clinically significant, haematological abnormalities are common in individuals with HIV/AIDS. These abnormalities may occur as a result of HIV infection itself, as sequelae of HIV-related opportunistic infections or malignancies or as a consequence of therapies used for HIV infection and associated conditions. HIV actually

infects the progenitor cells or causes them to function abnormally [2]. The consequences of haematological problems are twofold. First, they have major morbidity in themselves, adversely altering the patient's quality of life. Second, they hinder the treatment of both the primary viral infection and the opportunistic infections [3].

There is paucity of data from India on the haematological manifestation of HIV which prompted us to conduct this study using one of the most easily available investigations (complete haemogram). We also tried to evaluate the relationship between various haematological manifestations and CD4 counts, CD4 counts and ALC in a medical college-based observational study of HIV-infected adults attending R.C.S.M. Govt. Medical College, Kolhapur in 1 year (March 2015- March 2016).

## Subjects and Methods

HIV seropositive patients referred from ART centre of R.C.S.M Govt Medical College, Kolhapur for CBC to haematology department, central clinical laboratory (CCL) from March 2015 to March 2016 were included in this study, irrespective of their ART status. Written informed consent was obtained from all. Patients were excluded if they were less than 18 years of age or were pregnant or refused to become part of study.

After taking informed consent, detailed clinical history of every subject was recorded which included history of any opportunistic infection and treatment taken. Also, general and systemic examination was done. Haematological parameters (Hb, TLC, DLC, Platelet count, MCV, MCH, MCHC, HCT) were determined using the haematology auto analyzer CounCell-23 plus whereas the immunological parameter (CD4+ T cells) were assayed using the BD FACS Count system. Differential leucocyte count and patterns of anaemia were studied on peripheral blood smear stained with Leishman stain. ESR was carried out. Absolute lymphocyte count (ALC) was calculated as-

ALC (cells/cu.mm) = Differential lymphocytes (%) X TLC (cells/cu.mm)

The collected information was compiled in a predetermined proforma.

Anaemia was defined as haemoglobin <13g/dl (men) and <12g/dl (women), leucopenia as total leucocyte count <4000cells/µl, neutropenia as absolute neutrophil count <1000cells/µl, lymphopenia as absolute lymphocyte count <800cells/µl and thrombocytopenia as platelet count $<150\times10^3$ cells/µl.

The study was carried out after taking permission from the Institute's Ethical Committee and MSACS (Maharashtra State AIDS Control Soceity).

### *Statistics*

Descriptive statistics were expressed as percentage and mean±SD. Statistical tests used were chi-square test, Fisher's exact test and Mann-Whitney U test. Kolmogorov-smirnov analysis was used to assess the linearity of the data. Pearson's rank order correlation was used to assess the correlation between two parameters. ROC curve was plotted to assess the diagnostic significance of the parameters.

Sensitivity, specificity, positive predictive value and negative predictive value were used to assess the accuracy of diagnostic significance of ALC to predict CD4 count <200cells/µl at different levels. $P<0.05$ was treated as statistically significant. SPSS Vs. 16 (IBM Corp)® and Microsoft excel (Microsoft corp. pvt. ltd. ™) were used to perform the statistical analysis.

## Results

Total number of HIV seropositive patients presenting in CCL in one year in the prescribed period was 356. The number of cases meeting the inclusion critaeria were 235. 10 cases had insufficient data so they were excluded from the study. Hence, the sample size for study was 225.

Majority of HIV positive patients in our study belonged to 30-40 years age group (36.4%, n=50) followed by 40-50 years (29.3%, n=82). Out of the 225 subjects, 54.6% (n=123) were males and 45.3% (n=102) were females. Most of the patients in our study were receiving ART (91%, n=205) while 9% (n=20) patients were not receiving ART .

Prevalence of anaemia in the present study was found to be 54.6% (n=123). Commonest type of anaemia found was microcytic hypochromic anaemia (MHA) seen in 25.9% (n=58) of the cases followed by macrocytic anaemia (MA) in 17.3% (n=39) and normocytic normochromic anaemia (NNA) in 11.5%(n=26) cases. Among grades of anaemia, moderate anaemia was most common, present in 26.2% (n=59) cases followed by mild anaemia present in 21.3% (n=48)cases and severe anaemia in 7.1% (n=16) cases.

Table 1 summarizes the results of haematological parameters evaluated in 225 HIV infected individuals. Table 2 shows correlation of assessed parameters with CD4 count of study subjects.

Table 3 shows comparison of haematological parameters in subjects with different CD4 count. Table 4 depicts diagnostic significance of different levels of ALC for CD4 count<200cells/µl. Figure 1 shows correlation analysis of CD4 count and ALC. Figure 2 shows Receiver Operating Characteristic (ROC) curve displaying the result of sensitivity and false positive error rate of the same to determine best cut off value of ALC.

**Table 1:** Frequency (%) of study subjects having normal, decreased and increased values of haematological parameters

| Haematological parameter | Normal (%) | Decreased (%) | Increased (%) |
|---|---|---|---|
| Haemoglobin (g%) | 45.3 | 54.6 | - |
| TLC (cells/cu.mm) | 80.4 | 15.2 | 4.4 |
| Differential neutrophil (%) | 87.5 | 0 | 12.4 |
| Differential lymphocyte (%) | 44.4 | 53.3 | 2.3 |
| Differential monocyte (%) | 98.22 | 1.78 | 0 |
| Differential eosinophil (%) | 100 | 0 | 0 |
| Differential basophil (%) | 100 | 0 | 0 |
| Platelet count (cells/cu.mm) | 88.4 | 4.4 | 7.1 |
| MCV (fl) | 25.7 | 28.8 | 45.5 |
| MCH (pg) | 15.5 | 40.9 | 43.6 |
| MCHC (g/dl) | 20.9 | 32.4 | 46.7 |
| PCV (%) | 15.1 | 80.9 | 4 |
| ESR (mm/hr) | 28 | 0 | 72 |
| ALC (cells/cu.mm) | 43.1 | 56.9 | 0 |
| CD 4 (cells/µl) | 29.7 | 69.3 | 1 |

**Table 2:** Correlation of assessed parameters with CD4 count of study subjects

| Parameters | Mean | Pearson's r | $r^2$ | p Value |
|---|---|---|---|---|
| Age (years) | 39.4 ± 9.8 | 0.04 | 0.001 | 0.5 |
| Hb (g%) | 11.31 ± 2.23 | 0.32 | 0.12 | 0.02 |
| PCV% | 34.1 ± 4.9 | 0.02 | 0.0004 | 0.7 |
| MCV (fl) | 90.3 ± 13.05 | 0.41 | 0.21 | 0.02 |
| MCH (pg) | 30.5± 6.7 | 0.26 | 0.21 | 0.02 |
| MCHC (%) | 33.3 ± 3.65 | 0.1 | 0.1 | 0.17 |
| TLC (cells/µl) | 5874 ± 2031 | 0.02 | 0.005 | 0.9 |
| Platelet count (lakh cells/ µl) | 2.52 ± 0.64 | 0.06 | 0.004 | 0.3 |
| Differential neutrophil (%) | 63.3 ± 8.14 | -0.3 | 0.09 | <0.001 |
| Differential lymphocyte(%) | 22.8 ± 10.4 | 0.7 | 0.5 | <0.001 |
| Differential monocyte (%) | 2.48 ± 0.75 | 0.02 | 0.004 | 0.9 |
| Differential eosinophil (%) | 1.05 ± 0.23 | 0.03 | 0.0009 | 0.6 |
| Differential basophil (%) | 0.90± 0.55 | -0.01 | 0.0003 | 0.7 |
| ESR (mm/hr) | 14.25 ± 4.39 | 0.024 | 0.0006 | 0.7 |
| ALC (cells/µl) | | 0.49 | 0.24 | <0.0001 |

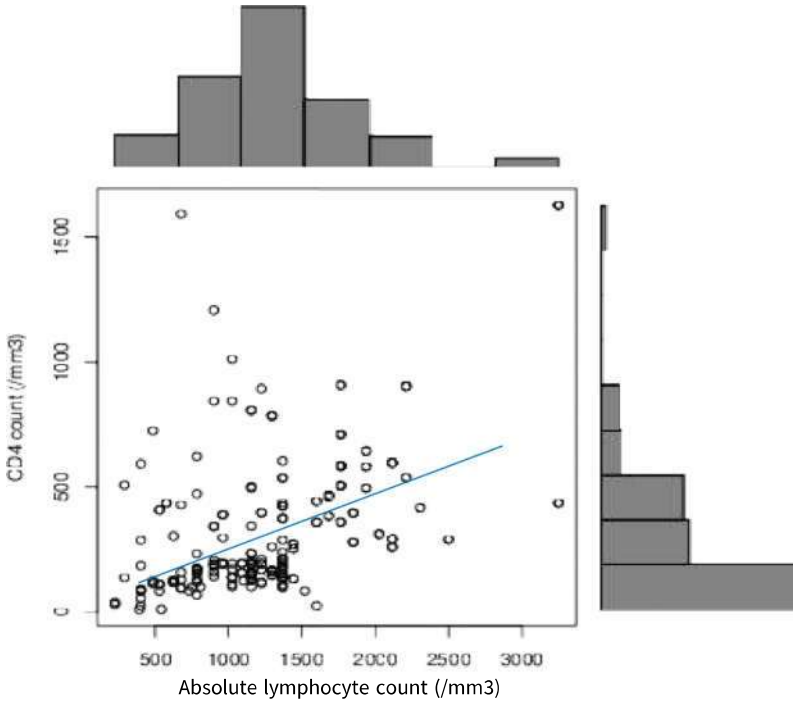


**Fig. 1:** Correlation analysis of CD4 count with absolute lymphocyte count (ALC) in cells/µl

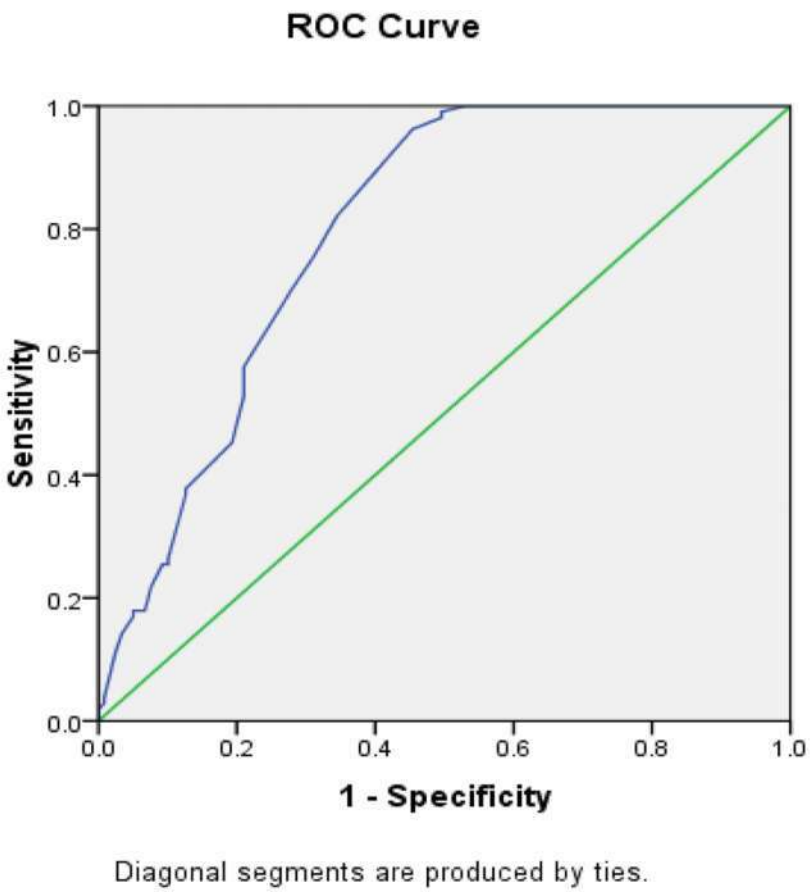


**Fig. 2:** ROC curve[1]
[1]Area under curve 79.6% i.e. 0.80

**Table 3:** Comparison of assessed parameters among different CD4 count groups of study subjects

| Haematological parameter | CD4<200 cells/cu.mm N (%) | CD4 200-350 cells/cu.mm N (%) | CD4 >350 cells/ cu.mm N (%) | P value |
|---|---|---|---|---|
| Mild anaemia (Hb-10-12g/dl) | 31(14%) | 6(3%) | 11(4%) | 0.01 |
| Moderate anaemia (Hb-7-10 g/dl) | 52(23%) | 2(1%) | 5(2%) | |
| Severe anaemia ( Hb- <7g/dl) | 6(3%) | 0(0%) | 10(4%) | |
| Leucopenia | 10(4%) | 11(5%) | 13(6%) | 0.003 |
| Lymphocytopenia | 93(41%) | 13(6%) | 22(10%) | 1.59 |
| Thrombocytopenia | 3(1%) | 5(2%) | 2(1%) | 0.01 |
| Decreased MCV | 37(16%) | 8(4%) | 20(9%) | 0.3 |
| Decreased MCH | 55(24%) | 10(4%) | 27(12%) | 0.03 |
| Decreased MCHC | 49(22%) | 6(3%) | 18(8%) | 0.0009 |
| Decreased PCV | 102(45%) | 26(12%) | 54(24%) | <0.0001 |
| Raised ESR | 77(34%) | 25(11%) | 60(27%) | 0.6 |
| Number of subjects in each group | 110 (49%) | 33 (15%) | 82 (36%) | |

N= Number of subjects out of total study population (x/225)

**Table 4:** Diagnostic significance of different levels of absolute lymphocyte count (ALC) for CD4 count <200 cells/µl

| ALC cut –off | Sensitivity | Specificity | Positive predictive value | Negative predictive value |
|---|---|---|---|---|
| ≤1000 | 52.8 | 89.0 | 28.3 | 80.5 |
| ≤1200 | 69.8 | 72.8 | 59.3 | 65.3 |
| ≤1400 | 96.2 | 54.6 | 89.4 | 50.1 |
| ≤1600 | 95.8 | 47.1 | 92.3 | 62.4 |
| ≤1800 | 100 | 45.6 | 87.2 | 64.7 |
| ≤2000 | 100 | 42.3 | 94.1 | 61.3 |

## Discussion

Haematological complications are a common cause of mortality in HIV infected patients. In different study settings, the prevalence of anaemia in persons with AIDS is different but it's more common than thrombocytopenia or leucopenia in patients with AIDS [4]. Cytopenias are most frequent during advanced stage of disease [5]. We evaluated 225 consecutive HIV seropositive patients who presented at Central Clinical Laboratory, RCSM GMC, Kolhapur, irrespective of their ART status. We also correlated the final haematological diagnosis of patients with CD4 count and assessed the utility of Absolute Lymphocyte Count (ALC) to predict CD4 count.

HIV-related anaemia generally is due to reduced red blood cell (RBC) production, secondary to Bone marrow suppression by HIV virus, due to cytokine production, alteration in the bone marrow microenvironment, nutritional deficiencies, increased RBC destruction, or a combination of these problems. Study subjects in our study had high frequency of low - Hb values 123 (54.6%); (p=0.02), MCV 65 (28.8%); (p=0.02), MCH 92 (40.9%); (p=0.02), MCHC 73 (32.4%) and PCV 182 (80.8%) compared to normal values (Table 1,2).

This confirms research by Tagoe DNA et al. (39.6%, p <0.001), Dikshit B et al. (65.5%, p =0.081), Attili SVS et al (74.6%, p=0.001), Ibeh BO et al. (76%) and Parinitha SS (84%) who also observed a drop in Hb levels. The prevalence of anaemia in our study is less when compared to other studies, it can be attributed to the fact that in our study out of 225, 205 patients were on ART and ART is known to improve anaemia [6-10].

The decrease in MCV (p=0.02), MCH (p=0.02), MCHC indicates that HIV positive patients experience microcytic hypochromic anaemia conditions which got confirmed by peripheral blood smear examination. This is in accordance with study conducted by Tagoe DNA et al and Dikshit B et al. Ibeh BO et al also observed decreased values of PCV in his study [6,7,9]. ART may cause macrocytosis and maybe the reason why MCV of HIV infected participants in this study was high 102 (45.3%). However, we did not find any patient with haemolytic anaemia.

Total leucocyte counts (TLC) of HIV+ patients were found to be reduced 34(15.2%) with mean of 5.87±2.03cells/cu.mm when compared to normal values (Table 1). This observation is similar in works by Tagoe DNA et al, Ibeh BO et al and Parinitha SS et al who observed leucopenia in 26.8%, 26.8% and 20.8% patients respectively. Leucopenia is known to increase the incidence of opportunistic infections in HIV patients [6,9,11].

Majority of HIV+ patients had normal neutrophil differential (%) values 197 (87.5%) resulting in a mean of 63.3±8.14% which is within the normal % (Table 1). However, 28(12.4%), were neutrophilic confirming study by Tagoe DNA et al who observed neutrophilia in 36.2% HIV+ patients (p=0.005). Neutrophilia can be because of bacterial infections HIV positive patients are prone to [6]. 120 (53.3%) cases in our study had significantly decreased lymphocyte differential(%) (p <0.001) and 128 (56.9%) cases had decreased absolute lymphocyte count (Table 1,2). A similar observation was made by Parinitha SS et al, Choi SY et al. who also reported lymphopenia in 65.2% and 25.7% cases respectively. Groenewald AJ et al. also observed significant lymphopenia in his study (p <0.0001) [10-12].

We found only 10 (4.4%) HIV patients having thrombocytopenia (mean-2.52±0.64 lakh cells/cu.mm) (Table 1). Attili SVS et al and Choi SY et al. reported prevalence of thrombocytopenia in 4.8% and 2.4% study subjects respectively. In none of our patients was it the presenting feature and none of them had significant bleeding [8,12]. However, thrombocytopenia is known to complicate HIV infection. Thrombocytopenia may be a result of increased platelets destruction or decreased platelet production in subjects not on antiretroviral treatment (ART).

The mean Erythrocyte sedimentation rate (ESR) of HIV+ patients was higher (14.25±4.39mm/hr) compared with the normal ESR values due to decreased erythrocyte count (anaemia) and haematocrit such that 162(72%) had high ESR values (Table 1, 2) agreeing with the work of other authors [6,9,10,13]. In a study by Smith EM et al, ESR determination in HIV infected patients was found to be a predictor of the development of AIDS and that ESR is important when coupled with a CD4 count of <500cells/ìL and an elevated b2-microglobulin in predicting the progressing to AIDS [14].

CD4 lymphocyte count is essential for assessment of immune status in HIV-infected persons as the pathogenesis of AIDS is largely attributed to a decrease in absolute CD4 cell counts. CD4 cell counts are the criterion for categorising HIV-related clinical conditions by CDC classification system for HIV infection [15]. In our study, mean CD4 count was 337.08±285.4cells/µl and 156 (69.3%) cases had low CD 4 count, a similar observation was made by other authors (Table 1,2) [6,7,10,12].

Haematological parameters were compared in three groups (Table 3). The number of cases with anaemia, leucopenia, and lymphopenia and raised ESR increased as CD4 cell counts of patients falls. Parinitha SS et al and Enawgaw et al reported similar result in their studies [10,16]. Anaemia, leucopenia and thrombocytopenia showed significant difference (indicated by P<0.05) between three groups with differing CD4 cell counts. Majority of the study subjects had CD 4 count<200 (49%), 15% had between 200 and 350, 36% had >350 cells/µl. Higher

prevalence of anaemia may be caused by direct and indirect effect of HIV infection (viral load), opportunistic infections, and toxicity of the drugs. Suppression of bone marrow and direct infection of T cells leads to lymphocytopenia as CD4 count decreases. This condition reduces the body's resistance to many opportunistic infections and the patient becomes more susceptible to bacterial infections and needs medical attention, the condition may become life-threatening. Thrombocytopenia probably increases as immunological incompetence worsens thus leading to increased risk of excessive bleeding. Also, ESR was significantly raised ($p<0.001$) in study by Parinitha SS et al which is in accordance with our study though ESR wasn't significantly raised in our study [10,16].

We found significant moderately positive correlation between ALC and CD4 cell count with r value 0.49, $R^2=0.24$, (p-value < 0.0001), 95%C.I.=0.39-0.59, DF=223 (Figure 1). Other studies from India, Kumarasamy N et al., Karanth SS et al., Kakar A et al., and Sreenivasan S et al., also found high degree of correlation between CD4 cell count and ALC count with r-value 0.744, 0.682, 0.714, 0.560 respectively [17-20]. A similar trend has also been suggested in studies from other parts of world, Daka D et al., and Angelo ALD et al., reported high correlation with r-values 0.398 and 0.581 respectively [21,22].

Many investigators from different countries and regions of the world are evaluating the usefulness of ALC as surrogate marker of a CD4 cell count less than 200cells/îl for HIV infected patients of different ethnicities to be of help in resource poor settings where facilities for CD4 count estimation are not available. In our study, we found that WHO's suggestion of ALC ≤ 1200/μl, had low sensitivity of 69.8% and specificity 72.8%; positive predictive value 92.3%; negative predictive value 62.4%. However, study by Karanth SS et al., and Obirikorang C et al., show higher sensitivity(73% and 72.22%) and specificity (100% and 100%), respectively, for ALC cut-off of 1200 cells/îl to predict CD4 cell count< 200 cells/μl [18-23]. This difference could be due to different ethnic, racial, epidemiological and socioeconomic factors.

According to our finding, ALC of ≤ 1400 cells/μl was more sensitive (96.2%) to predict CD4 cell count of <200 cells/μl (Table 4). Kumarasamy N et al., and Kakar A et al., also found ALC cut-off < 1400 cells/μl having sensitivity of 73%, 64.4% and specificity 88%, 91.1% respectively for predicting CD4 cell count of <200 cells/μl. Other studies from India and across the globe also suggested higher ALC cut-off for predicting CD4 cell count of <200 cells/μl [17-22].

In our study, ALC obtained a high diagnostic performance (Area Under Curve= 0.80) for predicting CD4 cell count less than 200 cells/μl with sensitivity of 96.2% and specificity of 54.2% at threshold of ≤ 1400 cells/μl(Figure 2).

HAART recovers neutropenia, lymphopenia, thrombocytopenia, anaemia. Relatively less changes in haemoglobin, PCV and platelet count in HIV seropositive patient could be an indication of low toxicity in patients on HAART. This is a prospective observational study, so we didn't estimate the survival in patients with and without anaemia. Also, a possibility of coexisting iron deficiency anaemia and megaloblastic anaemia couldn't be ruled out as serum ferritin and serum Vit B12/Folic acid level respectively were not done.

ART drug toxicities are common findings so accordingly the treatment should be modified after haematology investigation confirmation. Hence, routine monitoring of haematological parameters in patients with HIV/AIDS is recommended, to detect the abnormalities at the earliest, find the aetiology and treat appropriately. These measures will reduce the morbidity and mortality.

*Key Messages*

Anaemia is the most common haematological manifestation of human immunodeficiency virus (HIV) infection and acquired immunodeficiency syndrome. Anaemia, neutropenia, lymphocytopenia and thrombocytopenia are reversible by HAART therefore early diagnosis and treatment plays an important role in improving patients' survival and quality of life.

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