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Evolution of Hematobiochemical Profiles in Newly Diagnosed HIV Patients and HIV-TB Co-Infected Patients: Correlation with Immunological and Virological Status

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Background: CD4+ cells, HIV-1 plasma viral load (PVL), and IFN-γ have been observed to enhance susceptibility in TB infection/reactivation among HIV-1 infected people, leading to unusual clinical manifestations. HIV-TB co-infection is significant for immunological and virological response, making it a great clinical challenge for patient management. The objective of this study was to explore the correlation among various hematological and biochemical profiles with CD4+ count and PVL in order to decipher mechanisms of TB development or reactivation in HIV-infected patients.

Methods: In this cross-sectional study, we included 200 newly diagnosed treatment naïve HIV-1 infected patients, of which 118 were HIV-TB co-infected and 82 were HIV-alone. The CD4+ T count was determined using the BD FACS Count System, and the plasma HIV-1 viral load was estimated using the Abbott m2000 real-time platform. The hematobiochemical testing was performed on fully-automated analyzer ADVIA® 560 and Cobas® 501 Roche Diagnostics. Statistical software SPSS-2, Spearman correlation analysis was used for data analysis and a P-value less than 0.05 was considered statistically significant.

Results: Declined hemoglobulin level positively correlated with CD4 counts ($r = 0.229$; $p = 0.001$), and a negative correlation was observed with HIV-1 plasma viral load ($r = -0.171$; $p = 0.016$). Moreover, the CD4+ count and HIV-1 plasma viral load (PVL) were also correlated to anomalies such as thrombocytopenia, leucopenia, eosinophils, neutrophils, ESR, potassium, Albumin, globulin, SGOT, uric acid. Studies also found significantly higher absolute neutrophil count, ESR, and serum fasting blood sugar, creatine, uric acid, total bilirubin, globulin, and alkaline phosphatase in HIV-TB co-infected patients.

Conclusion and Recommendation: The initial value of Hb, ESR, absolute neutrophil counts, serum calcium, uric acid, and potassium can be used as an early indicator for active tuberculosis (TB) and as a substitute marker for the course of HIV disease, especially in areas with low resources.

Keywords: HIV, HIV-TB coinfection, CD4 count, plasma viral load, hematological parameters

Introduction

Human immunodeficiency virus or Acquired immunodeficiency syndrome (HIV/AIDS) is a prominent public health issue world wide. As per recent published data by World health organization (WHO), there are currently 39.9 million individuals worldwide who are living with HIV.[1] As per UNAIDS-2024 report 2,318,738 individuals in India currently infected with HIV, with 68,000 new cases in 2023.[2] Individuals with HIV frequently encounter tuberculosis (TB) as an

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opportunistic infection in areas with minimal resources.[3] The combination of HIV and TB is highly lethal, as they mutually exacerbate each other's disease progression. Additionally, TB is a primary factor contributing to mortality in individuals who are HIV-positive.[3] HIV-1-infected individuals are more susceptible to developing active TB compared to those who are HIV-negative. It has been observed that there is a decline in CD4 cells during HIV progression and is linked to a decrease in interferon-gamma production, which in turn increases the likelihood of TB reactivation.[4,5] Furthermore, the rising prevalence of (TB) has been linked to the occurrence of unusual clinical manifestations, as well as difficulties in diagnosing and treating TB among HIV-1 patients. In addition, there is a correlation between active (TB) infections and heightened immunological activation, which leads to an increase in the replication of HIV-1. Consequently, this accelerates the progression of HIV illness. The immunocompromised state of HIV-infected patients leads to an increased occurrence and severity of hematological abnormalities, which is a significant consequence. The cause could be attributed to the modification of the bone marrow associated with HIV.[4,5] Nevertheless, those who have not yet received Antiretroviral Therapy (ART-naïve), as well as those who are infected with HIV-1, commonly experience notable hematological abnormalities such as anemia, cytopenia, neutropenia, lymphopenia, and thrombocytopenia.[6] Global reports suggest that the occurrence of anemia in individuals infected with HIV varies from 6.3% to 84%. Among these reports, India has the highest prevalence, ranging from 38.8% to 84%. In addition, research investigations have reported a 65.2% occurrence of lymphocytopenia and an 18% occurrence of thrombocytopenia.[6,7] Multiple investigations have documented that tuberculosis alone is linked to alterations in hematological and biochemical parameters.[6–8] Irregularities in the distribution of certain biochemical markers such as liver enzymes, total protein, albumin, globulin, alkaline phosphatase, bilirubin, creatinine, blood urea, electrolytes, and blood sugar levels are frequently observed in individuals with HIV-1 infection, especially in those receiving ART.[8] A recent study indicated a significant decrease in hemoglobin (Hb), platelet count (PLT), and total leukocyte count (TLC) in HIV-infected persons relative to those uninfected with HIV. In the same study, biochemical indicators such as Albumin (Alb) were significantly reduced, while urea levels were found to be elevated in HIV patients. The author proposed that these hematobiochemical characteristics may serve as indicators for HIV disease progression, especially in resource-constrained environments.[9] The systemic characteristics of HIV infection can lead to many organ dysfunctions, including impairments in bone marrow. This organ is pivotal in hematopoiesis; hence, hematological abnormalities are commonly noted in HIV-infected people, regardless of the stage of HIV disease.[10] The etiology of HIV-related bone marrow abnormalities is intricate; nonetheless, it has been proposed that HIV's capacity to infect bone marrow mesenchymal stem cells is the principal factor responsible for the morphological and functional changes in the bone marrow of individuals with HIV infection, correlating with the severity of bone marrow and hematological abnormalities as HIV disease progresses.[10,11]

HIV-associated nephropathy is acknowledged as a notable comorbidity in HIV patients, with identified risk factors including advanced age, ethnicity, low CD4 counts, elevated viral load, diabetes, and hypertension.[12] Alfano et al proposed that HIV induces a cytopathic effect on renal parenchymal cells and mediates failure of nephrons through immune complexes.[13] Changes in liver enzymes have been commonly observed in HIV patients, potentially resulting from both direct and indirect inflammation of hepatocytes associated with HIV.[14] Pathania et al conducted a study revealing that 51.8% of HIV-infected patients exhibited abnormal liver function tests (LFTs). Nonetheless, the majority exhibit minor anomalies.A statistically significant connection was discovered between CD4 count and the albumin/globulin ratio.[15] A study conducted by Mata-Marín et al identified a robust positive association between blood AST levels and HIV viral load, alongside a modest correlation between serum ALT levels and HIV viral load.[14] In nations with limited resources, the outcomes and analysis of regular clinical laboratory tests, such as hematology and biochemistry parameters, play a significant role in evaluating the advancement of HIV illness along with following up the ART treatment response. Numerous studies have documented the progression of hematological and biochemical profiles in HIV.[6–9] The correlation between hematobiochemical markers and the immunological and virological status in HIV-TB co-infection vs HIV alone remains inadequately documented. This study aims to assess the hematological and biochemical changes and their relationship with immune system status (CD4 count) and virological state (HIV-1 PVL) in patients diagnosed with both HIV and TB, compared to those diagnosed solely with HIV.

Materials and Methods

The current cross-sectional study was conducted at the All-India Institute of Medical Sciences (AIIMS) New Delhi, India. We recruited 200 patients recently diagnosed with HIV-1 and without having a history of receiving (HAART) highly active antiretroviral therapy and (ATT) antituberculosis treatment. These patients were then separated into two groups. 118 patients showing signs of active tuberculosis in addition to HIV-1 infection (HIV-TB co-infected group) and 82 patients having HIV-1 infection without active tuberculosis (HIV-only group). A standardized questionnaire was utilized to gather demographic information, and signed consent was acquired from all patients prior to sample collection.

Inclusion and Exclusion Criteria

The inclusion criteria were age >18 years, HIV-1 tested positive (as per NACO guidelines), and no history of ART and ATT treatments. The Pregnancy, patients having serological evidence of HCV/HBV co-infection, history of chronic liver and renal diseases, malignancy, hemoglobinopathy, current and recent history of blood parasite infection, and not willing to participate were excluded.

Sample Collection, HIV Serology Testing, CD4 and HIV-1 Plasma Viral Load Estimation

A venipuncture procedure was used to acquire a blood sample of 10 to 12 mL. The sample was collected into various vacutainer vials, including K3-EDTA, sodium fluoride, and plain vials manufactured by Becton Dickinson. The plasma was extracted from the K3-EDTA tube, divided into 1 mL portions in 1.5 mL cryovials, and preserved at −80◦C for further estimating the viral load of HIV-1.

HIV serology testing was performed to detect HIV-specific antibodies in the patient's serum sample- as per NACO (National AIDS Control Organization) national HIV serology testing guideline. Three different enzyme immunoassay (EIA) or enzyme-linked immunosorbent assay (ELISA) based methods were used, and one test was based on the principle of differentiation between HIV-1 and HIV-2.[16]

The CD4+ T count was determined using the BD FACS Count System. The plasma HIV-1 viral load was estimated by Abbott m2000 real-time platform, following the instructions provided by the manufacturer.

Laboratory Testing of Hematological and Biochemical Parameters

The biochemistry parameters, including RFT, LFT, and fasting blood sugar (using a sodium fluoride vial), were analyzed using a fully-automated analyzer (Cobas® 501, Roche Diagnostics) within two hours of sample collection. The analysis followed the approved standard operating procedure (SOP) and include routine quality control measurements for each parameterThe hematology profile, specifically the complete blood counts (CBC), was performed using a K3-EDTA vial on a hematology analyzer called ADVIA® 560, manufactured by Siemens Healthineers. Before analyzing the samples, the vial was placed on a mechanical roller for five minutes to ensure proper mixing. Additionally, daily routine quality control specimens, including normal, low, and high samples, were run to confirm the quality of the results. Erythrocyte sedimentation rate (ESR) was conducted using a semi-automated analyzer utilizing Westergren's method.

Anemia was identified as hemoglobin levels below 13 g/dL for males and below 12 g/dL for females. Were as Leukocytopenia: WBC count < 4000/µL, Neutropenia: Neutrophil count < 1500/µL, Lymphocytopenia: Lymphocyte count < 800/µL, Eosinophilia: Eosinophil count > 440/µL, Thrombocytopenia: Platelet count < 1.5 lakh/µL. Normal fasting blood sugar: 70–100 mg/dL Prediabetic: 100–125 mg/dL Diabetic: >126 mg/dL Hypoglycemia: <70 mg/dL (WHO).

Statistical Analysis

Statistical software SPSS-21 was used to analyse the data and the data visualization software GraphPad Prism 5.0. The clinical and sociodemographic data were evaluated using measures of central tendency (mean), dispersion (standard deviation), and percentages. Parametric and non-parametric tests were used to compare groups. Spearman correlation analysis was used to assess the relationship among HIV-1 viral load, CD4 count, as well as biochemical and hematological markers. *p value* less the 0.05 was considered statistical significant.

Results

Clinical Characteristics

The present investigation included of 200 individuals who were recently diagnosed with HIV-1. 82 patients who did not have active tuberculosis and were not receiving antiretroviral therapy (HIV without TB), referred to as group I. The remaining 118 participants who had active tuberculosis and were ART naïve (HIV with active TB), referred to as group II (Table 1).

Average age of participants in group I (HIV without TB) was 34.73 ± 5.78 years, and 65.9% of men were present. Furthermore, individuals reported smoking rates of 7.3% and 17.1% for the present and past periods, respectively. Heterosexual contact is the main way HIV is transmitted, accounting for 87.8% of cases. Nevertheless, in group II (HIV with active TB), the average age of patients was 36.23 ± 8.4, and a majority of 81.4% were men. The reported prevalence of past smoking history was 51.7%, indicating a significant proportion of individuals with a history of smoking. Both groups predominantly observed heterosexual mode of HIV transmission, accounting for 82.4% of cases. There was a significant prevalence of anemia among male patients (82.3%) who had both HIV and TB, in contrast to those with HIV alone (33.3%). In addition, anemia was detected in 86.4% of female patients with co-infection of HIV and TB and in 78.6% of female patients with HIV alone. The prevalence of leukocytopenia was higher in patients having both infections

Table 1 Baseline Characteristics of HIV+TB- and HIV+TB+ Patients

Variables	HIV+TB- (n=82)	HIV+TB+ (n=118)
Age (Years)	34.73 ± 5.78	36.23 ± 8.4
BMI, Kg/m2	21.73 ± 1.66	18.94 ± 2.18
Gender, number (%)		
Male	54 (65.9%)	96 (81.4%)
Female	28 (34.1%)	22 (18.6%)
Smoking History (%)		
Current	6 (7.3%)	7 (5.9%)
Past	14 (17.1%)	61 (51.7%)
Never	62 (75.6%)	50 (42.4%)
HIV Risk Factor, number (%)		
Heterosexual	72 (87.8%)	109 (82.4%)
Homosexual/MSM	6 (7.3%)	4 (3.4%)
Other	04 (4.9%)	5 (4.2%)
Anemia (%)		
Male	18 (33.3%)	79 (82.3%)
Female	22 (78.6%)	19 (86.4%)
Leukocytopenia (%)	6 (7.3%)	16 (13.6%)
Neutropenia (%)	2 (2.4%)	4 (3.4%)
Lymphocytopenia (%)	Nill	18 (15.3%)
Eosinophilia (%)	18 (23%)	38 (32.2%)
Thrombocytopenia (%)	10 (12.2%)	19 (16.1%)
Fasting Blood Sugar Level (%)		
Normal **(%)**	46 (56.1%)	76 (64.4%)
Prediabetics **(%)**	Nill	11 (9.3%)
Diabetics **(%)**	Nill	Nill
Hypoglycemia **(%)**	36 (43.9%)	31 (26.3%)

Notes: - = Negative, + = Positive, n= Number of participants, % = Percent, MSM= Males who have Sex with Males, BMI=Body mass index, Mean ± SD.

(13.6%) compared to only HIV infection (7.3%). Neutropenia was identified in 2.4% of patients with HIV alone and in 3.4% of participants with HIV-TB co-infection. Lymphocytopenia was absent in HIV-alone individuals, but 15.3% of HIV-TB co-infected patients exhibited lymphocytopenia. Additionally, we observed a higher frequency of eosinophilia (32.2%) and thrombocytopenia (16.1%) in individuals co-infected with HIVand TB compared to only HIV patients, where 23% exhibited eosinophilia and 12.2% had thrombocytopenia. The normal fasting blood sugar was observed in 56.1% of individuals with HIV alone and 64.4% in patients with HIV-TB co-infection.

Conversely, prediabetes (9.3%) was exclusively observed in patients having HIV and TB. Furthermore, the occurrence of hypoglycemia was more in individuals with HIV infection alone (43.9%) compared to those with both HIV and TB coinfection (26.3%). Neither of the groups had diabetes.

Our investigation revealed a substantial variance in the median CD4 count in the patients co-infected by HIV and TB (143 (181.4±138.2)) and patients with HIV alone (320 (336.9±110.4)). The CD4 count of co-infected patients was notably lower (*p=<0.0001*). In addition, there was a notable increase in the median HIV-1 viral load in patients with both infections (583,421 hIV-1 RNA copies/mL) compared to individuals with HIV alone (104,306 hIV-1 RNA copies/mL) (Figure 1).

Comparison of Hematological Parameters Among HIV+TB- and HIV+TB+ Patients

We conducted a comparison of the baseline hematological parameters between newly diagnosed HIV-infected patients and those with active TB. When studying patients with active tuberculosis, it was shown that those who were also infected with HIV had considerably lower levels of hemoglobin compared to those who were only infected with HIV (10.66 ± 1.906 vs 12.30 ± 2.32, *p= 0.0001*). The HIV+TB- group had a substantially lower absolute neutrophil count (ANC) of 3272.41 ± 1.018 compared to the HIV+TB+ group, which had an ANC of 4452.12 ± 3.47 (*p= 0.001*). Additionally, we noticed a substantial increase in the (ESR) among those in HIV+TB+ group (55.60 ± 29.10) compared to only HIV (24.95 ± 13.07) *(p=0.0001*). Although the platelet count (PLT), total leucocyte count (TLC), absolute eosinophil count (AEC), absolute lymphocyte count (ALC), and absolute monocyte count (AMC) were comparable, no significant was observed among these two groups (Table 2).

Comparison of Biochemical Parameters Among HIV+TB- and HIV+TB+

We conducted a comparison of the baseline biochemistry parameters between newly diagnosed HIV-infected patients having no evidence of active tuberculosis and HIV-infected patients having evidence of active tuberculosis (Table 3). The study found that the plasma fasting sugar was considerably higher in the patients who had both HIV and tuberculosis (79.95 ± 14.78) compared to the group of individuals who were only HIV positive (72.63 ± 9.79) *(p=0.0001)*. The HIV+TB+ group had a notably elevated level of creatinine (0.84±0.20) than HIV+TB- patients

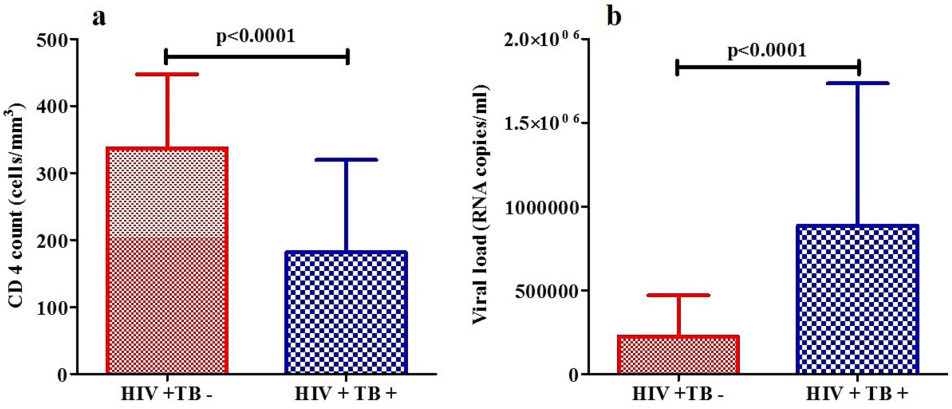

Figure 1 CD4 count and HIV-1 viral load comparison among HIV and HIV-TB co-infected patients. In figure (**A**), CD4 count revealed a substantial variance in the median CD4 count in the patients co-infected by HIV and TB (143 (181.4±138.2)) and patients with HIV alone (320 (336.9±110.4)). The CD4 count of co-infected patients was notably lower (p=<0.0001). Figure (**B**) showed the notable increase in the median HIV-1 viral load in patients with both infections (583,421 hIV-1 RNA copies/mL) compared to individuals with HIV alone (104,306 hIV-1 RNA copies/mL).

Table 2 Hematological Parameters Among HIV+TB- and HIV+TB+ Patients

Hematological Parameters (mean ± SD)	*Reference Values	HIV+TB- (n=82)	HIV+TB+ (n=118)	p-value
Hemoglobin (g/dL)	12–15	12.3 ± 2.32	10.66 ± 1.90	**0.0001**
Platelet count (Lakhs/μL)	2.5–4.5	2.13 ± 0.67	5.70 ± 0.69	0.179
Total leucocyte count (×10^9/L)	4000 −11,000	6326.83 ± 1.94	7301.86 ± 5.37	0.073
Absolute neutrophil count (×10^9/L)	1500 −8000	3272.41 ± 1.01	4452.12 ± 3.47	**0.001**
Absolute lymphocyte count (×10^9/L)	800–5000	2174.59 ± 874.72	1851.59 ± 1.64	0.074
Absolute eosinophil count (×10^9/L)	40–440	426.80 ± 865.69	511.90 ± 1.40	0.597
Absolute monocyte count (×10^9/L)	160–1000	434.29 ± 188.72	385.24 ± 390.72	0.240
Erythrocyte sedimentation rate (mm/hr)	0–20	24.95 ± 13.07	55.60 ± 29.102	**0.0001**

Notes: Mean ± SD, [*Institutional Reference values approved by All-India Institute of Medical Sciences, New Delhi, India; these values may vary with laboratories] (Bold indicates the statically significant p-value less than 0.05).

Table 3 Biochemical Profiles Among HIV+TB- and HIV+TB+ Patients

Clinical Biochemistry Parameters (mean ±SD)	#Reference Range	HIV+TB- (n=82)	HIV+TB+ (n=118)	p value
BSF (mg/dL)	70–100	72.63 ± 9.79	79.95 ± 14.78	**0.0001**
Urea (mg/dL)	10–50	22.12 ± 7.16	23.01 ± 7.28	0.395
Creatinine (mg/dL)	0.5–1.8	0.77 ± 0.19	0.84 ± 0.20	**0.022**
Calcium (mg/dL)	8.5–10.5	8.55 ± 0.51	8.33 ± 0.86	**0.025**
Phosphate (mg/dL)	2.5–4.5	3.67 ± 0.68	3.73 ± 0.61	0.466
Uric Acid (mg/dL)	2–7.4	4.98 ± 1.19	5.95 ± 2.06	**0.0001**
Sodium (mEq/L)	130–149	139.61 ± 4.02	137.74 ± 5.07	**0.004**
Potassium (mEq/L)	3.5–5	4.53 ± 0.54	4.16 ± 0.56	**0.0001**
Total Bilirubin (mg/dL)	0.2–1.20	0.44 ± 0.22	0.56 ± 0.36	**0.003**
Total Protein (g/dL)	6.0–8.0	7.71 ± 0.57	8.92 ± 8.88	0.146
Alb (g/dL)	3.5–5.0	4.45 ± 0.64	3.68 ± 0.60	**0.0001**
Glb (g/dL)	2–3.5	3.28 ± 0.74	4.03 ± 0.82	**0.0001**
SGOT (IU/L)	0–50	42.10 ± 30.48	51.19 ± 37.64	0.061
SGPT (IU/L)	0–50	38.56 ± 21.31	41.57 ± 29.92	0.408
ALP (IU/L)	80–240	229.05 ± 106.91	281.13 ± 202.13	**0.019**

Notes: # Institutional Reference values approved by All-India Institute of Medical Sciences, New Delhi, India; these values may vary with laboratories. Bold indicate the statically significant p-value less than 0.0.
Abbreviations: BSF, Blood Sugar Fasting; Alb, albumin; Glb, globulin; SGOT, Serum Glutamic Oxaloacetic Transaminase; SGPT, Serum Glutamic Pyruvic Transaminase; ALP, alkaline phosphatase.

(0.77 ± 0.19) *(p=0.022)*. The HIV+TB+ patients had a substantially higher level of uric acid (5.95±2.06) than HIV +TB- participants (4.98 ± 0.19) *(p=0.0001)*. HIV+TB+ participants showed significantly higher total bilirubin level (0.56 ± 0.36) compared to the HIV+TB- group (0.44 ± 0.22) (*p=0.003*). Similarly, the HIV+TB+ participants had a significantly higher globulin level (4.03 ± 0.82) than the HIV+TB- patients (3.28 ± 0.74) *(p=0.001)*. Additionally, the HIV+TB+ group had a significantly higher level of alkaline phosphatase enzyme (281.13 ± 202.13) compared to the HIV+TB- participants (229.05 ± 106.91) *(p=0.019)*. The HIV+TB+ patients exhibited significantly lower levels of electrolytes, specifically calcium (8.33 ± 0.861 than HIV+TB- group 8.55 ± 0.519, *p=0.025*), and sodium (137.74 ± 5.07 compared to the HIV+TB- group 139.61 ± 4.024, *p=0.004*). The HIV+TB+ group exhibited a notably lower potassium level of 4.16 ± 0.56 compared to the HIV+TB- group with a level of 4.53 ± 0.54 *(p=0.0001)*. Similarly, the HIV+TB+ group showed a considerably lower albumin level of 3.68±0.60 compared to the HIV+TB- group with a level of 4.45 ± 0.647 *(p=0.0001)*. There were no notable variations in measures such as urea, phosphate, total protein, SGOT, and SGPT liver enzyme between the two groups.

Correlation of CD4 Count with Baseline Hematological and Biochemical Parameters

A correlation study was conducted to examine the relationship between CD4 cell counts and hematological and biochemical parameters (Table 4, Figures 2 and 3). We found positive association among Hb (r = 0.229, *p= 0.001*), TLC (r = 0.163, p= 0.021), and ALC (r = 0.464, *p= 0.0001*). An inverse association was observed with ESR (r = −0.435, *p= 0.0001*).

Additionally, a positive association was observed between potassium (r = 0.191, *p= 0.007*) and albumin (r = 0.502, *p= 0.0001*). A significant negative correlation was found with total bilirubin (r = −0.157, *p= 0.027*), globulin (r = −0.363, *p= 0.0001*), and serum SGOT (r = −0.192, *p= 0.006*).

Correlation of Plasma Viral Load with Baseline Hematological and Biochemical Parameters

Furthermore, a Spearman correlation analysis examined the relationship between viral load and hematological and biochemical markers (Table 5, Figures 4 and 5). The study found a clear and statistically significant positive relationship between viral load and platelet counts (r = 0.14, p= 0.04) and ESR (r = 0.37, *p= 0.0001*).

An evident inverse relationship was established between Hb (r = −0.17, *p= 0.016*) and ALC count (r = −0.16, *p= 0.01*). The biochemical parameters showed significant positive correlations with viral load. Specifically, uric acid (r = 0.26, *p= 0.0001*), total bilirubin (r = 0.17, *p= 0.015*), total serum protein (r = 0.20, p= 0.004), globulin (r = 0.33, *p= 0.0001*), SGOT (r = 0.21, *p= 0.003*), and alkaline phosphate (r = 0.17, *p= 0.013*) all exhibited significant positive associations with viral load. An inverse relationship was identified between viral load and potassium (r = 0.16, *p= 0.018*), albumin (r = −0.25, *p= 0.0001*).

Table 4 Corelation Between CD4 Count Along Hematological and Biochemical Parameters

Variables	Correlation Coefficient (r)	p value
Hematological parameter		
Hemoglobin (Hb)	0.229	**0.001**
Platelet	0.050	0.480
Total leucocyte count (TLC)	0.163	0.021
Absolute neutrophil count	−0.047	0.509
Absolute lymphocyte count (ALC)	0.464	**0.0001**
Absolute eosinophil count	0.081	0.253
Absolute monocyte count	0.113	0.112
Erythrocyte sedimentation rate (ESR)	−0.435	**0.0001**
Clinical biochemistry		
BSF	−0.119	0.093
Urea	0.000	0.998
Creatinine	−0.018	0.795
Calcium	0.113	0.112
Phosphate	−0.060	0.398
Uric Acid	−0.065	0.362
Sodium	0.113	0.110
Potassium	0.191	**0.007**
Total Bilirubin	−0.157	**0.027**
Total Protein	−0.049	0.489
Alb	0.502	**0.0001**
Glb	−0.363	**0.0001**
SGOT	−0.192	**0.006**
SGPT	−0.077	0.279
ALP	−0.131	0.065

Note: Bold indicates the statically significant p-value less than 0.05.

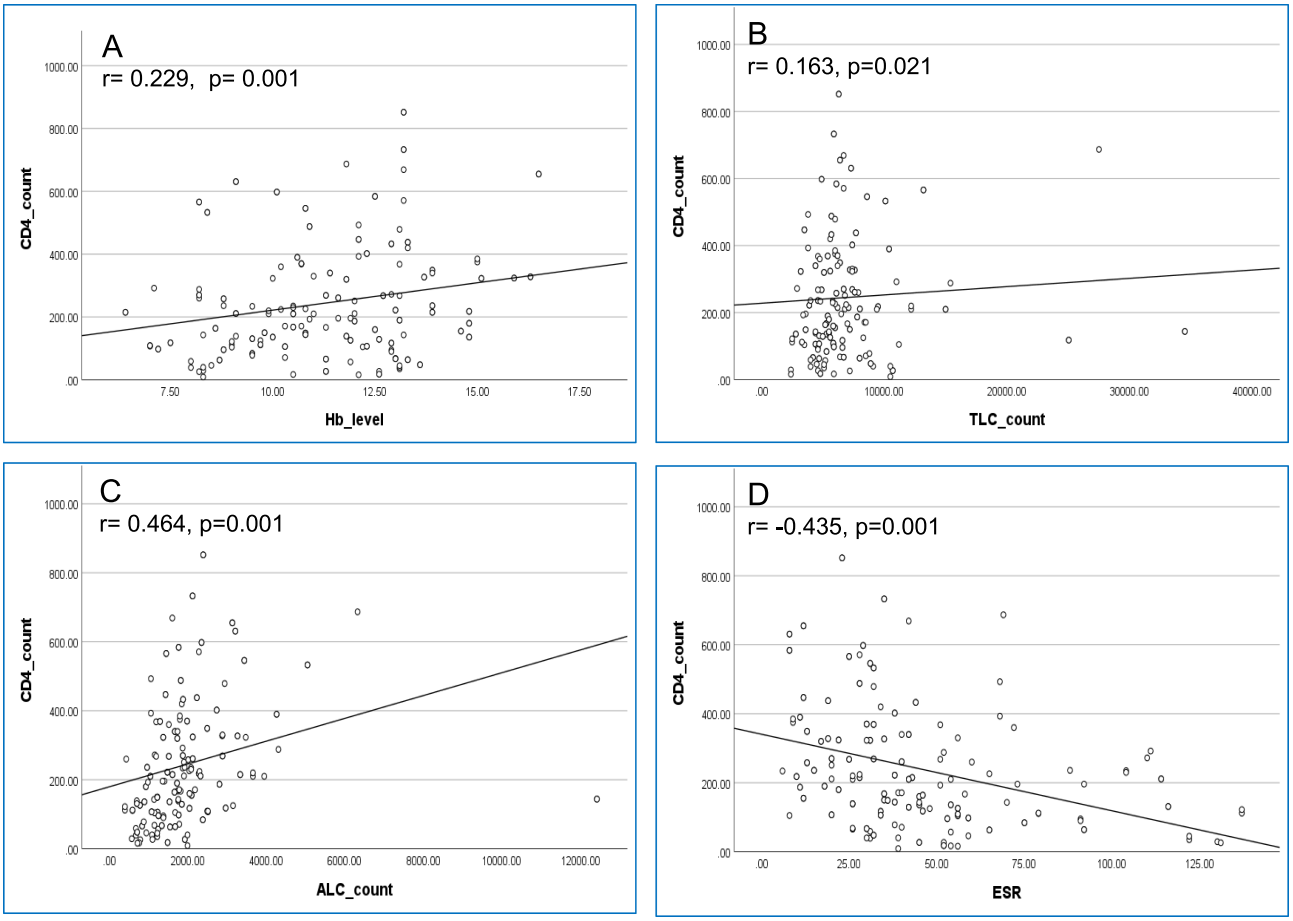

Figure 2 CD4 count showed the significant correlation with with hematological profiles- (**A**), Hb level [r=0.229, *p=0.001*], (**B**) TLC count [r=0.163, *p=0.021*], (**C**) ALC count [r=0.464, *p=0.001*], (**D**) ESR [r= −0.435, *p=0.001*].

Discussion

Mycobacterium Tuberculosis infection is a prevailing opportunistic infection among patients infected with HIV in resource-limited countries such as India. It is a significant cause of death in this population.[17] Manifestation of tuberculosis in HIV patients exhibits variability and primarily relies on their immunological and virological condition. Tuberculosis (TB) indirectly facilitates the reproduction of HIV and hence accelerates the rate at which HIV progresses.[18] Previous research has consistently shown a notable decline in CD4 count and an increase in HIV-1 PVL in similar investigations.[19] In addition, the hematological and biochemical parameters were comparable with the findings of Kirchhoff et al and Marchionatti et al, indicating decreased hemoglobin levels in both HIV+ and HIV-TB+ individuals.[5,6] However, a higher frequency of anemia was identified among the female participants, both in those co-infected with HIV-TB (86.4%) and those with HIV alone (78.6%). In addition, the prevalence of anemia was higher in male patients with HIV-TB co-infection (82.3%) as compared to male patients with HIV alone (33.3%). People living with HIV experience significant abnormalities in their blood cell counts, including low levels of white blood cells (leukocytopenia), low levels of lymphocytes (lymphocytopenia), and low levels of platelets (thrombocytopenia).[20] Similarly, the population co-infected with HIV and TB showed higher levels of all hematological parameters compared to patients infected with only HIV. This finding aligns with the study conducted by Bisetegn and Ebrahim (2021), where they observed a prevalence of 11.91% (in ART-naïve individuals) and 5.95% (in ART-experienced individuals) for thrombocytopenia, as well as a prevalence of 17.31% for leucopenia among people living with HIV.[20] HIV-associated anemia is complex, encompassing both virus-related and non-virus-related variables. The direct effect of HIV can impair hematopoiesis by causing bone marrow defects through the infection of mesenchymal stem cells, dysregulating iron

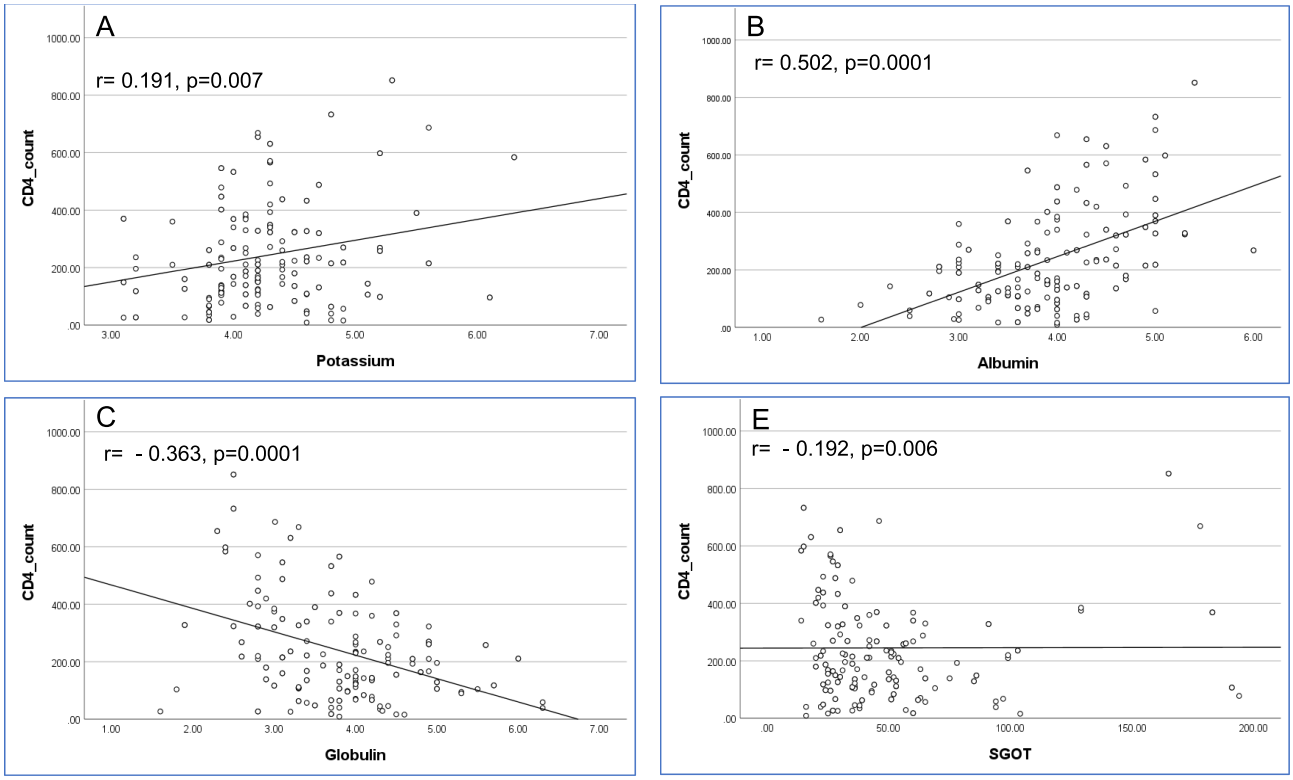

Figure 3 CD4 count indicating significant correlation with biochemical parameters (**A**) Potassium [r=0.191, p=0.007], (**B**) Albumin [r=0.502, *p=0.0001*], (**C**) Globulin [r= −0.363, *p=0.0001*], (**D**), SGOT [r= −0.192, *p=0.006*].

transport, and reducing iron availability for hemoglobin synthesis; however, the interplay between HIV, anemia, and iron metabolism is intricate. Similarly, in anemia of chronic illnesses, iron is sequestered within macrophages, thereby diminishing the iron accessible for erythropoiesis.[10,11] Moreover, chronic inflammation in HIV-infected persons may stimulate hepcidin synthesis, an iron-regulatory hormone; elevated hepcidin levels can lead to reduced iron absorption

Table 5 Correlation Between PVL Along Hematological and Biochemical Parameters

Variables	Correlation Coefficient (r)	p-value
Hematological parameter		
Hemoglobin (Hb)	−0.171	**0.016**
Platelet	0.143	**0.044**
Total leucocyte count	0.012	0.869
Absolute neutrophil count	0.079	0.268
Absolute lymphocyte count	−0.166	**0.019**
Absolute eosinophil count	0.035	0.618
Absolute monocyte count	−0.097	0.173
Erythrocyte sedimentation rate	0.377	**0.0001**
Clinical biochemistry parameters		
BSF	0.122	0.085
Urea	0.098	0.167
Creatinine	0.040	0.578

(*Continued*)

Table 5 (Continued).

Variables	Correlation Coefficient (r)	p-value
Calcium	−0.120	0.090
Phosphate	−0.011	0.875
Uric Acid	0.264	**0.0001**
Sodium	−0.091	0.202
Potassium	−0.167	**0.018**
Total Bilirubin	0.173	**0.015**
Total Protein	0.202	**0.004**
Alb	−0.257	**0.0001**
Glb	0.331	**0.0001**
SGOT	0.210	**0.003**
SGPT	0.101	0.154
ALP	0.176	**0.013**

Note: Bold indicates the statically significant p-value less than 0.05.

and sequestration, thus contributing to anemia in those with HIV[10,21] Moreover, factors such as nutritional deficiency, co-infection with opportunistic infections, immunocomplex-mediated hemolysis of red blood cells, and oxidative stress resulting from HIV infection can lead to redox imbalance, adversely impacting red blood cell survival and functionality,

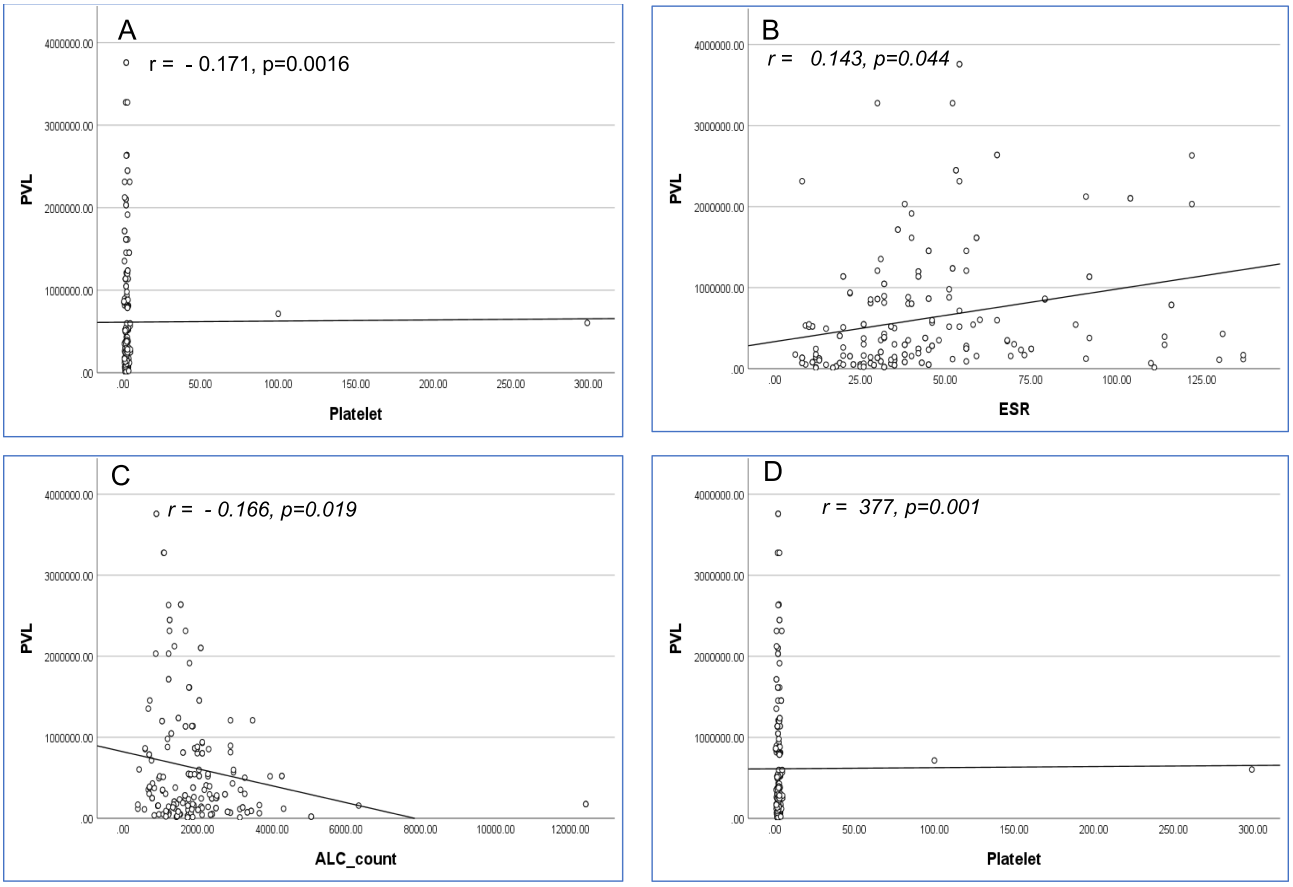

Figure 4 PVL count showed the significant correlation with hematological parameters (**A**) Hemoglobin [r= −0.171, *p=0.0016*], (**B**) Platelet [r=0.143, *p=0.044*], (**C**) Absolute lymphocyte count [r=- 0.166, *p=0.019*], (**D**) ESR [r= 377, *p=0.001*].

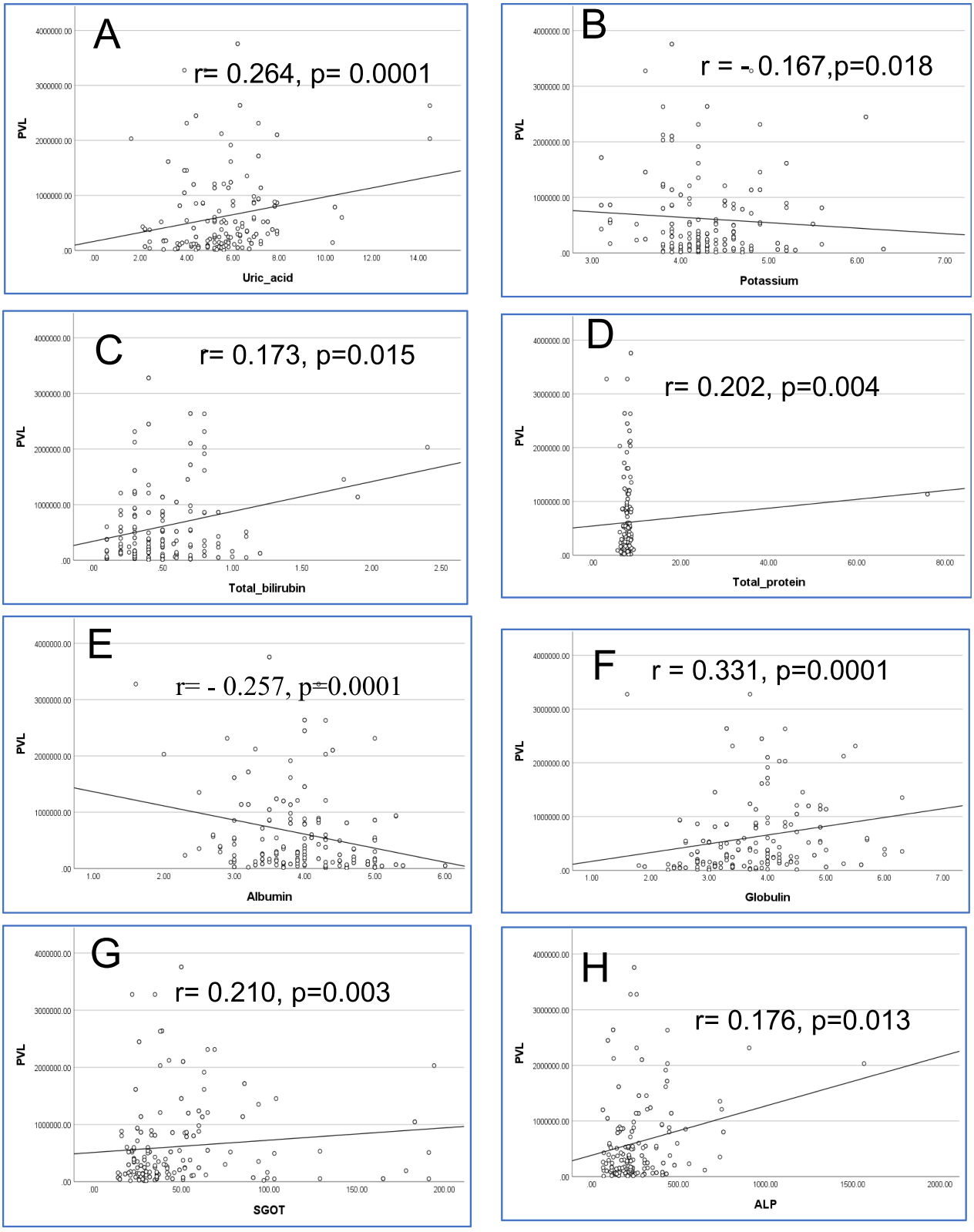

Figure 5 PVL count showed the significant correlation with biochemical parameters (**A**) Uric acid [r=0.264, *p=0.0001*], (**B**) Potassium [r= −0.167, *p=0.018*], (**C**) Total Bilirubin [r=0.173, *p=0.015*], (**D**) Total protein [r= 0.202, *p=0.004*], (**E**) Albumin [r= −0.257, *p=0.0001*], (**F**) Globulin [r=0.331, *p=0.0001*], (**G**) SGOT [r= 0.210, *p=0.003*]. (**H**) ALP [r= 0.210, *p=0.003*].

ultimately resulting in hemolysis and contributing to the development of anemia. Opportunistic infections, chronic inflammation, and the detrimental effects of certain antiretroviral therapy medications, such as zidovudine, may significantly contribute to the onset of anemia in individuals with HIV/AIDS.[21]

In addition, the progression of HIV disease and the occurrence of opportunistic infections have various effects on HIV-infected individuals, particularly in terms of severe hematological abnormalities. These abnormalities often include increased eosinophils, neutrophils, and ESR (erythrocyte sedimentation rate), as documented in studies[22–24] It was reported that the prevalence of diabetes among people living with HIV (PLWH) was 14% in urban settings and 0.9% in rural regions.[25] However, the studied patients did not have a recorded history of diabetes. However, elevated plasma fasting blood sugar levels, serum creatinine, uric acid, total bilirubin, globulin, and alkaline phosphate enzyme (ALP) were detected. Liusha, Namakandoet al, individuals with HIV who also have kidney impairment are more prone to having an active tuberculosis infection compared to those with HIV alone.[26]

Hypocalcemia was frequently seen as a prevalent electrolyte abnormality in people living with HIV (PLWH).[27] Nevertheless, we observed a notable decrease in serum calcium levels in patients co-infected with HIV and TB compared to those without TB. Calcium may contribute to the development of M. tuberculosis infection. However, a study conducted by Malik, Z. A. et al proposed that M. tuberculosis hinders the calcium signaling mediated by CR and suggests that this modification of macrophage activation had a role in preventing the fusion of phagosomes with lysosomes. This, in turn, facilitates the survival of mycobacteria within the host cells.[28] Comparable to our findings, a study conducted by Afridi, Hassan Imran et al reported markedly reduced concentrations of calcium in the serum samples of AIDS patients with tuberculosis.[29] HIV-infected patients with advanced stages of the disease are more prone to electrolyte disorders due to altered renal physiology, which could be caused by HIV-associated nephropathy effect, chronic inflammation, gastrointestinal opportunistic infections, and endocrinological disruption, which may be consequences of several clinical conditions such as diarrhea, fever, vomiting, and polyuria ultimately responsible of loss of electrolyte. Additionally, these conditions require prompt medical interventions, including antibiotics and antiviral drugs, which may accelerate electrolyte disorders.[30]

In addition, individuals with HIV show widespread swelling of the lymph nodes and an abnormal increase in the production of multiple types of antibodies, which impacts the ratio of albumin to globulin.[31] Similarly, Dey et al reported that individuals with HIV exhibited hypergammaglobulinemia. Significant changes were observed in these values, except for serum total protein levels, in individuals who were co-infected with HIV and TB.[32] Following the correlation study, we noted a positive link between CD4 count and hematological parameters such as hemoglobin, total leukocyte, and absolute lymphocyte counts. Conversely, a negative correlation was found between CD4 count and ESR. Similarly, a study conducted by Abdollahi, Alireza et al reported a significant association between CD4 and total lymphocyte count as well as hemoglobin.[33] Furthermore, there was a notable positive association between CD4 count and biochemical measures such as potassium and albumin. Conversely, bilirubin, globulin, and SGOT exhibited negative correlations with CD4 count. Contrary to our results, Tinarwo, Partson et al found no significant correlation between CD4 cell count and serum potassium level.[34] Continuation with our findings, Samuel M. et al demonstrated a correlation between reduced CD4 counts and decreased serum albumin levels.[35] The HIV-1 viral load exhibited a favorable association with platelet count and ESR while demonstrating a negative association with hemoglobin and absolute lymphocyte counts. Similarly, a study conducted by Al-Mughales J. A. et al demonstrated a negative association between HIV-1 viral load and hemoglobin, and absolute lymphocytes were documented. Unlike our findings, the study reported that platelet count is negatively associated with HIV-1 VL.[36] The serum total bilirubin is a highly dependable indicator for individuals with liver failure.[37] In a study by Huang, Huihuang et al investigated a correlation between the virological state of HIV-1 infection and the occurrence of liver damage. The study found that there was a substantial link between the baseline HIV-1 viral load and the incidence of liver damage. In addition, it was reported that patients with elevated levels of HIV-1 viral load were more prone to liver damage in comparison to individuals with lower levels of HIV-1 viral load.[38]

The study found a positive correlation with HIV-1 viral load and total proteins, globulin, and ALP. However, there was a negative correlation with albumin. This negative correlation may be attributed to the association between HIV-1 plasma viral loads and liver dysfunction. However, there is ample evidence to support the fact that both patients infected with HIV alone and patients co-infected with HIV and TB exhibited polyclonal hyperglobinopathy. In agreement with our

finding, a study conducted by Peluso MJ et al demonstrated that patients with elevated first plasma HIV-1 virus load exhibited greater initial SGOT levels.[39] AIDS patients frequently experience hypokalemia, with documented prevalence rates ranging from 5% to 53%.[30] Previous studies suggested that hypokalemia among HIV-infected patients is mainly caused by gastrointestinal potassium losses triggered by intestinal infections, resulting in excessive diarrhea.[40,41] The findings of our study revealed an inverse relationship between HIV-1 viral load and serum potassium levels. The link between high plasma HIV-1 viral loads and diarrhea-causing intestinal opportunistic parasite infections may be attributed to the former being a risk factor for the latter.[42]

Limitation

Due to the cross-sectional study, there may be certain limitations. One issue was the lack of initial measurements for some hematological and biochemical markers. Moreover, the absence of data about peripheral blood pictures, iron studies, Packed cell volume (PCV), MCV, MCH, MCHC, Glomerular filtration rate (GFR), and immunoglobulin levels may potentially restrict the scope of the study. Obtaining urine and stool samples would have been optimal for detecting proteinuria, obtaining a more comprehensive assessment of kidney function, and analyzing the stool for the presence of gastrointestinal parasite infection. Furthermore, the study did not incorporate any individuals who were considered normal and healthy and who did not have HIV. Also, the present study was not able to extrapolate the findings to the whole community/population due to the small sample size.

Conclusion and Recommendations

This study revealed notable differences in hematobiochemical parameters between patients with HIV-TB and those with HIV alone; specifically, hemoglobin, serum calcium, sodium, and albumin levels were significantly lower, while absolute neutrophil count, erythrocyte sedimentation rate, fasting blood sugar, creatinine, uric acid, total bilirubin, globulin, and alkaline phosphatase levels were significantly higher in HIV-TB co-infected individuals. Consequently, baseline hematobiochemical analytes may serve as preliminary indicators for HIV disease progression and tuberculosis co-infection, especially in resource-constrained settings. Furthermore, CD4 counts exhibited a substantial positive connection with hemoglobin levels, total lymphocyte count, serum potassium, and albumin. Conversely, plasma viral load had a markedly favorable connection with platelet count, erythrocyte sedimentation rate, uric acid, total bilirubin, total protein, globulin, serum glutamic-oxaloacetic transaminase, and alkaline phosphatase. Consequently, these analytes may serve as surrogate markers for HIV disease progression or as alternatives to HIV-1 viral load testing, particularly in low-income countries lacking access to viral load testing facilities. Hence, incorporating baseline hematological and biochemical routine indicators can assist in the clinical care of both HIV-TB coinfection and HIV-alone patients. Both groups typically exhibited low levels of hemoglobin (Hb) or anemia. We also noted a substantial decrease in hemoglobin (Hb) levels among HIV-infected patients who had active tuberculosis (TB). Additionally, it was shown that hemoglobin levels exhibited a statistically significant ($p= 0.016$) positive association with CD4 counts and a negative correlation with HIV-1 plasma viral load. Data uniquely establishes that the initial level of hemoglobin (Hb) can be used as an early indicator for active tuberculosis (TB) and as a substitute surrogate marker for HIV disease progression, especially in areas with low resources. Moreover, HIV-related anemia has various sources; thus, effective therapy necessitates a comprehensive strategy. This may involve promptly commencing effective antiretroviral therapy, choosing the most suitable treatments for opportunistic infections, and executing targeted nutritional measures, including supplementation of iron, protein, electrolytes, and other micronutrients. Moreover, the precise molecular mechanisms underlying anemia in HIV and the influence of HIV infection on iron metabolism remain inadequately elucidated; consequently, rigorous fundamental research and additional longitudinal studies are necessary to clarify this intricate interaction and to develop more effective therapeutic and nutritional strategies.

Ethical Clearance

The study received approval from the institutional ethics committee of the All-India Institute of Medical Sciences, New Delhi (IESC/T-88/01.02.2013). Participants informed consent was taken before the commencement of study and study was carried out in accordance to Declaration of Helsinki.

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Disclosure

The authors report no conflicts of interest in this work.

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