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Cross-Sectional Insights into Hematobiochemical Alterations in HIV Patients Receiving ART and Co-Infected with Viral Hepatitis in Pakistan

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HIV/AIDS, Antiretroviral therapy, Viral hepatitis, Co-infections, Hematological parameters, Biochemical alterations

ABSTRACT

HIV has become a significant public health concern in Pakistan. It shares modes of transmission with hepatitis B (HBV) and hepatitis C (HCV), and the co-infection of either or both viruses may exacerbate the HIV progression and complicate antiretroviral therapy (ART) outcomes. The present study aimed to compare hematological and biochemical profiles among HIV-positive individuals on ART and living with and without co-infection of HBV and HCV, to better understand the systemic impact of viral co-infections in a Pakistani population. This cross-sectional study was conducted from July to November 2024 on 100 HIV-positive patients receiving ART across various districts of Punjab. Participants were also screened through serological testing for HBV and HCV. Hematological parameters such as cluster of differentiation 4 (CD4) count, RBC, hemoglobin (Hb), WBC, and platelet (PLT) counts were assessed using standard analyzers, while biochemical markers were tested using enzymatic assays. Descriptive statistics, one-way ANOVA with Tukey's post-hoc test and regression analyses were used to determine group-wise differences. Among all groups, patients co-infected with both HBV and HCV exhibited the lowest mean CD4 count (224 ± 30.6/µL, $p < 0.01$), Hb (7.35 ± 0.17 g/dL, $p < 0.001$), RBC (3.52 ± 0.085 $\times 10^6$/µL, $p < 0.001$), and PLT count (154 ± 7.47 $\times 10^3$/µL, $p < 0.001$). Liver enzymes were significantly elevated in co-infected patients (ALT, 277 ± 13.6 U/L; AST, 281 ± 13.3 U/L; ALP, 260 ± 13.9 U/L all $p < 0.001$) compared to mono-infected individuals. Mono-infected patients had comparatively preserved immune and liver function. Co-infection with HBV and HCV in HIV patients on ART is associated with greater immune suppression and liver dysfunction, necessitating integrated management and routine monitoring for improved outcomes.

INTRODUCTION

The human immunodeficiency virus (HIV) is among the most significant infections of the twenty-first century (Meganck and Baric, 2021). It affects not only individuals but also families, communities, and society as a whole. HIV has been a growing global concern for the last forty years, with an estimated 38.6 million people affected worldwide (Payagala and Pozniak, 2024). The current state in Pakistan shows significant cause for concern. Pakistan has documented multiple outbreaks of HIV throughout the last twenty years as the country's HIV infection rate reached 210,000 individuals (Aizaz *et al*., 2023). The AIDS-related deaths have surged by 405%, leading to an incidence-to-mortality ratio of 3.33 (El-Jamal *et al*., 2024). The most affected populations include people who inject drugs (PWIDs), men who have sex with men (MSMs), sex workers, prisoners, and transgender individuals, as seen in recent outbreaks such as the one in Larkana (Raza *et al*., 2024).

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Contaminated syringes are the main route of HIV transmission in Pakistan, with the prevalence of HIV among PWIDs reaching 27.2% (Yaqub *et al*., 2021). HIV prevalence currently stands at 5.6% among MSM populations and 7.2% in transgender populations throughout the world. Among 30,192 people screened in 2019 researchers detected a 2.9% HIV positivity rate while 80% of positive cases affected people older than 15 years (Summer, 2021).

HIV causes significant hematological abnormalities. The virus primarily targets CD4+ T lymphocytes but also affects adjacent hematopoietic cells, resulting in hematological disorders such as anemia, neutropenia, and lymphopenia (Mandania, 2024). As HIV progresses, these abnormalities become more frequent and severe. Thrombocytopenia, for instance, can present early in the disease course in 3–12% of asymptomatic individuals (Raadsen *et al*., 2021). Furthermore, opportunistic infections and antiretroviral therapy (ART) may contribute to cytopenia and exacerbate metabolic disorders, including anemia (Obeagu and Kanu, 2024).

Co-infection with viral hepatitis further complicates the clinical picture of HIV patients. Patients who have both HIV and HBV together show deteriorating liver health and greater hepatotoxicity risks when they use ART treatments which fail to cover HBV effectively (Arora *et al*., 2021). Patients who take FTC monotherapy without addition of tenofovir often develop HBV resistance and reduced liver inflammation during successful immune restoration (Cheng *et al*., 2021). As a result, the WHO recommends an ART regimen containing at least two agents with HBV-suppressive activity, such as 3TC, TDF (or FTC), and efavirenz, as the preferred first-line treatment for HIV/HBV co-infection (Xu *et al*., 2021). Similarly, co-infection with the Hepatitis C Virus (HCV) is common due to shared routes of transmission with HIV. Globally, approximately 130 million people are currently infected with HCV (Shahriar *et al*., 2022). HIV/HCV co-infection increases the hepatic damage resulting in increased risk of hepatocellular carcinoma, and compromises the effectiveness of ART. These co-infections are major factors in morbidity and mortality among people living with HIV (Anejo-Okopi *et al*., 2021).

Despite the growing burden of HIV and its co-infections in Pakistan, there is limited data on the hematological and biochemical impacts of these co-infections in patients receiving ART. This cross-sectional study aimed to investigate the patterns of hematological (CD4 count, RBC, WBC, hemoglobin, platelets) and hepatic function biomarkers in HIV-positive individuals currently receiving ART and living with HBV and HCV co-infections in the Punjab, Pakistan.

MATERIALS AND METHODS

Sample size

A target sample size of hundred patients (n = 102) was calculated using epitool online calculator (https://epitools.ausvet.com.au/) considering human population in Punjab (127.7 million) (Pakistan, 2023), prevalence of HIV in Punjab as 0.04%, and selecting a desired absolute precision of 5% with a 99% confidence interval (Majeed *et al*., 2023).

Study population and sampling technique

This cross-sectional study was conducted at Institute of Punjab AIDS control program (PACP) Lahore. In the current study, HIV-positive patients selected using a stratified purposive sampling method from different region of the Punjab, Pakistan during July 2024 to November 2024. Eligible individuals included HIV-infected patients above 18 years of age who were receiving ART from different areas of Punjab from healthcare facilities receiving ART services. The data regarding demographic variable of the individuals was also recorded. All the samples were collected in compliance with the guidelines set by the Institutional Review Committee for Biomedical Research (No. 301/IRC/BMR, Dated: 07/11/2024). In addition, written informed consent was taken from all patients stating that data will be used for scientific publication. Approximately, 3 ml of whole blood sample was collected into K_2 EDTA-coated and clot activator vials, respectively and kept at 4 ºC for the further procedures.

HIV viral load estimation using real-time q-RT PCR

Plasma was separated from whole blood samples through centrifugation at 10000 rpm for 20 min using microfuge. Briefly, 10µL of plasma sample was loaded in the Cobas 5800 System that was used for automated viral load (per µL of plasma) estimation by following the standard procedures prescribed by the manufacturers (Tschäpe *et al*., 2022).

Screening of viral hepatitis

Screening of HBV was performed by using commercially available kit (Determine™ HBsAg 2, Alere Medical Co. Ltd, Chiba-ken, Japan). Approximately, 50 µL specimen for the test, and results was interpreted within 15 to 30 min (Kang *et al*., 2023). The Biolin™ (Abbott Diagnostics Korea Inc.) *in-vitro* rapid assay used to detect HCV specific antibody in the blood of the individuals as per manufacturers instruction.

Hematobiochemical profiling

Peripheral blood samples that were collected in EDTA

tubes and processed within 2 days for CD4 cells count. CD4+ T-lymphocyte counts were determined using the Alere Pima™ point-of-care testing (POCT) system. Each sample was processed within 3 h of collection. A measured volume of blood was loaded into a Pima™ Cartridge and inserted into the Pima™ Analyzer, which provided CD4 count results within approximately 20 min (Rathunde *et al*., 2014). Red cell indices including RBC (red blood cells), WBC (white blood cells), PLT (platelets), HCT (hematocrit), HGB (hemoglobin) were evaluated using Sysmex XP-100 analyzer (Ahmad *et al*., 2025).

Biochemical parameters

Biochemical parameters including alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin, total protein and albumin were estimated using standard procedures previously reported by Ahmad *et al*. (2023).

Statistical analysis

The data was initially compiled in MS Excel version 2021. Descriptive univariate analysis (percentages along with 95% confidence intervals) was performed to present the frequency distribution of the variables. Normality of the data was tested using Shapiro-Wilk test. One-way ANOVA with Tukey's post hoc test was used to compare the value of hematobiochemical parameters among the viral hepatitis co-infected and HIV mono-infected groups. Simple linear regression model was used to assess the impact of co-infection on these parameters. Chi-square test with Monte-Carlo simulations at 5000 bootstraps was performed to check the association of various symptoms with the co-infections. Analysis was performed in RStudio version 2024.12.1. All statistical tests were two-tailed, and a p-value < 0.05 was considered statistically significant.

RESULTS

Demographic analysis

A total of 69% of participants identified as male, and 2% as transgender. Median age of patients was 31.5 years (IQR; 15.75). In terms of duration since diagnosis, 44% had been diagnosed for 7–12 months, 38% for more than 25 months, and 18% for 13–24 months. Half of the participants were from the Lahore district (50%), with smaller proportions from other districts including Faisalabad (7%, 95% CI: 3.43–13.75), Sargodha (6%, 95% CI: 2.78–12.48), Bahawalpur (5%), Muzaffargarh and Rahim Yar Khan (each 4%) districts. Univariate analysis of the marital status, 54% of respondents were married, 39% were single, 6% were divorced, and 1% were widowed. Educational attainment varied with in the individuals, with 38% being uneducated, 32% having completed primary education, and 22% had secondary education (95% CI: 15–31.07). Only a smaller proportion had completed their higher secondary (4%), bachelor's (3%), or master's degrees (1%). The vast majority of participants (95%) reported no history of living or visiting abroad. Regarding the possible modes of HIV exposure, 37% had reported unprotected sexual contact with a sex worker, 35% reported sharing needles, 17% had a history of blood transfusion and 11% were men who have sex with men (MSM). Approximately, 51% were employed, while 49% were unemployed (Table I). However, bivariate analysis of the history of exposure and presence of co-infections did not reveal a significant association of the exposure routs (χ^2; 6.317, df; 9, p-value; 0.708).

Table I. Sociodemographic characteristics, HIV exposure history, and clinical background of HIV-positive patients on ART in Punjab, Pakistan (n = 100). Percentages are presented with 95% confidence intervals.

Variable	Percent (95% CI)
Gender	
Female	29% (21.01 - 38.54)
Male	69% (59.37 - 77.22)
Transgender	2% (0.36 - 7)
Duration since diagnosed	
13-24 months	18% (11.7 - 26.67)
25+ months	38% (29.1 - 47.79)
7-12 months	44% (34.67 - 53.77)
District of residence	
Bahawalnagar	4% (1.57 - 9.84)
Bahawalpur	5% (2.15 - 11.18)
Bhakkar	1% (0.05 - 5.45)
Faisalabad	7% (3.43 - 13.75)
Gujjranwala	3% (0.82 - 8.45)
Gujrat	2% (0.36 - 7)
Jhang	2% (0.36 - 7)
Kasur	1% (0.05 - 5.45)
Lahore	50% (40.38 - 59.62)
Layyah	2% (0.36 - 7)
Multan	3% (0.82 - 8.45)
Muzzaffargarh	4% (1.57 - 9.84)
Pakpattan	1% (0.05 - 5.45)
Rahim yar khan	4% (1.57 - 9.84)

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Variable	Percent (95% CI)
Rawalpindi	1% (0.05 - 5.45)
Sargodha	6% (2.78 - 12.48)
Sheikhupura	3% (0.82 - 8.45)
Toba Tek singh	1% (0.05 - 5.45)
Marital status	
Divorced	6% (2.78 - 12.48)
Married	54% (44.26 - 63.44)
Single	39% (30.02 - 48.8)
Widow	1% (0.05 - 5.45)
Education	
Bachelors	3% (0.82 - 8.45)
High secondary	4% (1.57 - 9.84)
Masters	1% (0.05 - 5.45)
Primary	32% (23.67 - 41.66)
Secondary	22% (15 - 31.07)
Uneducated	38% (29.1 - 47.79)
Living/visit abroad	
No	95% (88.82 - 97.85)
yes	5% (2.15 - 11.18)
History of disease exposure	
Blood transfusion	17% (10.89 - 25.55)
Sharing needle	35% (26.36 - 44.75)
Unprotected sex with sex worker	37% (28.18 - 46.78)
MSM	11% (6.25 - 18.65)
Occupation	
Job	51% (41.35 - 60.58)
Unemployed	49% (39.42 - 58.65)

HIV viral load estimation and hematological analysis

Comparison of the mean HIV viral load revealed the highest (Log_2 copies/mL; 17.7 ± 0.375), compared to lower levels in the HBV-positive (Log_2 copies/mL; 16.4 ± 0.674), HCV co-infected (Log_2 copies/mL; 17 ± 0.764), and HIV mono-infected individuals (Log_2 copies/mL; 16.1 ± 0.686). CD4 counts were significantly lower in the HBV and HCV co-infected group (224 ± 30.6 / µL) compared to the HIV mono-infection group (461 ± 58 /µL), reflecting greater immune suppression. The HIV mono-infected group had the significantly highest CD4 count overall, with intermediate values in the HBV co-infected (323 ± 25.4/µL) and HCV co-infected (349 ± 64.4) groups. Similarly, white blood cell (WBC) counts were significantly lower in the dual co-infected group (4.49 ± 0.243 $\times 10^3$/µL) compared to the HCV co-infected group (5.95 ± 0.475 $\times 10^3$/µL), while HBV co-infected and mono-infected individuals showed intermediate but statistically comparable values. Hemoglobin and red blood cell levels also revealed the dual co-infected group had the lowest Hb (7.35 ± 0.169 g/dL) and RBC (3.52 ± 0.0853 $\times 10^6$/µL) levels. In contrast, the mono-infected group showed the highest Hb (10 ± 0.693 g/dL) and RBC (4.77 ± 0.339 $\times 10^6$/µL) values. Platelet counts were also significantly reduced in all co-infected groups compared to the mono-infected group (276 ± 20.8 $\times 10^3$/µL), with the HBV and HCV co-infected group having the lowest mean PLT count (154 ± 7.47 $\times 10^3$/µL) (Fig. 1).

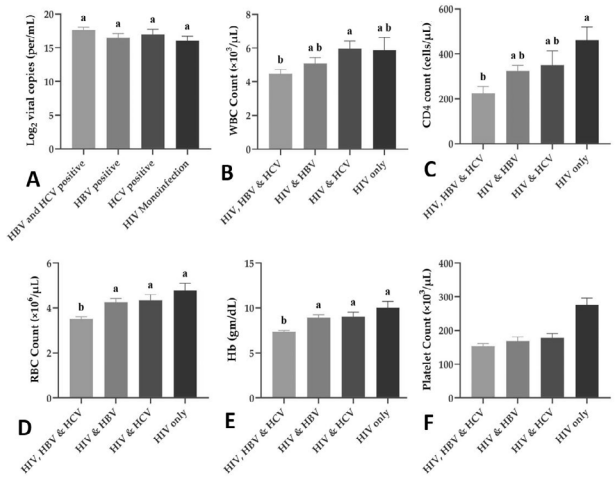

Fig. 1. Comparative analysis of hematological and virological parameters among HIV-positive patients with and without HBV and HCV co-infections. The four study groups include: HIV mono-infected (patients infected only with HIV), HIV+HBV (patients co-infected with hepatitis B virus), HIV+HCV (patients co-infected with hepatitis C virus), and HIV+HBV+HCV (patients co-infected with both HBV and HCV). Panels show: (A) HIV viral load expressed as log_2 viral copies per mL, (B) white blood cell (WBC) count ($\times 10^3$/µL), (C) CD4+ T cell count (cells/µL), (D) red blood cell (RBC) count ($\times 10^6$/µL), (E) hemoglobin concentration (Hb, g/dL), and (F) platelet count ($\times 10^3$/µL). Data are presented as mean ± standard error (SE). Groups with different superscript letters (A, B) differ significantly ($p < 0.05$), indicating statistical differences between the groups.

Serum biochemical analysis

Analysis of liver function parameters showed marked elevations among HIV-positive individuals co-infected with HBV and/or HCV. Total bilirubin levels were significantly ($p<0.05$) higher in all co-infected groups such as dual co-infected (1.75 ± 0.0805 mg/dL), HBV co-infected (1.68 ± 0.106 mg/dL), and HCV co-infected (1.6 ± 0.134 mg/dL) compared with the HIV mono-infected group (0.873 ± 0.09 mg/dL). Results regarding hepatic enzymes also showed that the dual co-infected group

exhibited the highest ALT (277 ± 13.6 U/L), AST (281 ± 13.3 U/L), and ALP (260 ± 13.9 U/L) levels. The HBV co-infected group showed moderately elevated enzyme levels (ALT: 206 ± 11.5 U/L; AST: 153 ± 8.12 U/L; ALP: 161 ± 13.3 U/L). On the other hand, the HCV co-infected group showed lower values but significantly ($p<0.05$) higher than those in HIV mono-infection (Fig. 2).

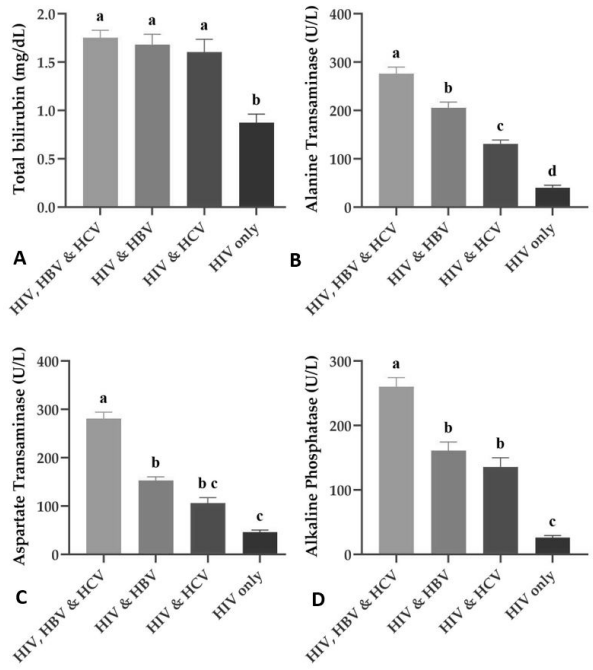

Fig. 2. Comparative analysis of hepatic function biomarkers among HIV-positive patients with and without hepatitis co-infections. The groups include: HIV mono-infected (patients infected only with HIV), HIV+HBV (co-infected with hepatitis B virus), HIV+HCV (co-infected with hepatitis C virus), and HIV+HBV+HCV (co-infected with both HBV and HCV). Panels show: (A) total bilirubin (mg/dL), (B) alanine transaminase (ALT, U/L), (C) aspartate transaminase (AST, U/L), and (D) alkaline phosphatase (ALP, U/L). Bars represent mean ± standard error of the mean (SEM). Groups labeled with different superscript letters (a, b, c, d) indicate statistically significant differences between the groups ($p < 0.05$).

Compared to HIV mono-infection, co-infection with both HBV and HCV was associated with the most pronounced alterations in hematological and liver function parameters. CD4 counts were significantly lower in the HBV and HCV co-infected group ($\beta = -237.7$, $p < 0.01$), whereas no significant changes were observed in the HBV ($\beta = -138.4$, $p>0.05$) or HCV ($\beta = -112.1$, >0.05) co-infected groups. Similarly, WBC levels declined significantly in the HBV+HCV group ($\beta = -1.385$, $p < 0.05$), but remained statistically nonsignificant in the other groups. Hemoglobin and RBC counts were both markedly reduced in the triple co-infected group (Hb: $\beta = -2.69$, $p < 0.001$; RBC: $\beta = -1.255$, $p < 0.001$), with non-significant decreases in the HBV and HCV co-infections alone. Platelet counts were significantly lower across all co-infected groups, with the most substantial reduction observed in HBV+HCV ($\beta = -122.1$, $p < 0.001$), followed by HBV ($\beta = -106.8$, $p < 0.001$) and HCV ($\beta = -98.2$, $p < 0.001$). Liver enzymes and total bilirubin were consistently elevated in all co-infected groups, with the highest increases seen in the HBV+HCV group (TB: $\beta = 0.877$, $p < 0.001$; ALT: $\beta = 235.9$, $p < 0.001$; AST: $\beta = 235.1$, $p < 0.001$; ALP: $\beta = 234.3$, $p < 0.001$) (Table II).

Table II. Linear regression analysis showing the impact of HBV and HCV co-infections on hematological and biochemical parameters in HIV-positive patients on ART, compared to HIV mono-infection. Beta (β) coefficients represent the mean difference in each parameter relative to the reference group (HIV only). Asterisks indicate levels of statistical significance: *p < 0.05, **p < 0.01, ***p < 0.001, ns = not significant.

	HBV and HCV co-infection	HBV co-infection	HCV co-infection
CD4 count (per mL)	-237.7**	-138.4ns	-112.1ns
WBC ($\times10^3/\mu L$)	-1.385*	-0.778ns	0.08ns
Hb (gm/dL)	-2.69***	-1.141ns	-1.036ns
RBC ($\times10^6/\mu L$)	-1.255***	-0.511ns	-0.431ns
PLT ($\times10^3/\mu L$)	-122.1***	-106.8***	-98.2***
TB (mg/dL)	0.877***	0.809***	0.731***
ALT (U/L)	235.9***	165.3***	90.3**
AST (U/L)	235.1***	106.9***	60.6*
ALP (U/L)	234.3***	135.3***	109.4***

The bivariate analysis of clinical symptoms between HIV mono-infected individuals and those co-infected with viral hepatitis (HBV and/or HCV) revealed that most symptoms did not differ significantly across groups based on chi-square test results ($p > 0.05$) except chronic diarrhea. A statistically significant association was found between co-infection status and chronic diarrhea, with a notably higher prevalence of chronic diarrhea (51.69%) was reported in HIV patients with viral hepatitis co-infection compared to those with mono-infection (18.18%) ($p = 0.036$). For other symptoms, including reoccurring pyrexia, progressive weight loss, cough, chronic fatigue, neuropathy, and paresthesia, no significant associations with co-infection status were observed (all p-values >0.05).

Although reoccurring pyrexia (61.8%) and progressive weight loss (59.55%) were numerically more common among co-infected individuals but the differences were not statistically significant. Neurological symptoms such as neuropathy and paresthesia were relatively rare and did not show significant variation between groups (Table III).

Table III. Comparison of clinical symptoms between HIV mono-infected and viral hepatitis (HBV and/or HCV) co-infected patients. Data represent the frequency and percentage of patients with and without symptoms in each group. Statistical significance was determined using the Chi-square test; p-values < 0.05 were considered significant.

	Absent	Present	P value
Reoccurring pyrexia			
Mono-infection	6 (54.55%)	5 (45.45%)	0.30
Viral hepatitis coinfections	34 (38.2%)	55 (61.8%)	
Progressive weight loss (>10%)			
Mono-infection	5 (45.45%)	6 (54.55%)	0.75
Viral hepatitis coinfections	36 (40.45%)	53 (59.55%)	
Cough			
Mono-infection	7 (63.64%)	4 (36.36%)	0.50
Viral hepatitis coinfections	47 (52.81%)	42 (47.19%)	
Chronic diarrhea (> 1 month)			
Mono-infection	9 (81.82%)	2 (18.18%)	0.036
Viral hepatitis coinfections	43 (48.31%)	46 (51.69%)	
Chronic fatigue (> 1 month)			
Mono-infection	8 (72.73%)	3 (27.27%)	0.44
Viral hepatitis coinfections	54 (60.67%)	35 (39.33%)	
Neuropathy			
Mono-infection	11 (100%)	0 (0%)	0.42
Viral hepatitis coinfections	84 (94.38%)	5 (5.62%)	
Paresthesia			
Mono-infection	11 (100%)	0 (0%)	0.37
Viral hepatitis coinfections	83 (93.26%)	6 (6.74%)	

DISCUSSION

Understanding regarding the incidence of viral hepatitis coinfections among individuals living with HIV is important for the policymakers and public health stakeholders in the development of effective strategies for prevention, treatment, and disease control (Simões *et al*., 2021). However, in resource-constrained settings such as in this study, the extent and clinical impact of hepatitis B and C virus coinfections in HIV-positive populations is currently inadequately reported (Adepoju, 2022). To our knowledge, the present study was first one to investigate the hematological and biochemical alterations in HIV-positive individuals receiving antiretroviral therapy, with a particular focus on those co-infected with viral hepatitis in Pakistan. A total of 100 samples were analyzed to assess red blood cell count, hemoglobin, platelet levels, and liver function markers across different HIV infected individuals on ART with co-infection statuses (HBV, and HCV). Additionally, the prevalence of symptoms like fever, weight loss, and diarrhea was evaluated to assess their clinical association with these laboratory abnormalities.

The socio-economic factors play a significant impact on limiting disease awareness. Targeting the enhancement of education is necessary for disease understanding and better treatment (Li, 2019). Education levels observed in the present study further support the link between low health literacy and high-risk exposure. A significant proportion of (38%) of participants was uneducated, and only 3% had attained a bachelor's degree or higher. The majority of participants were young adults (median age; 32), possibly due to social and behavioral vulnerabilities. Male patients were disproportionately affected and accounted for 69% of the study population. This is consistent with previously reported national trends where men are more frequently involved in high-risk behaviors such as drug injection and unprotected sex (Rabold *et al*., 2021). Our findings are consistent with Pakistan's ongoing HIV burden, where key affected populations include men who have sex with men (MSM), people who inject drugs, and transgender individuals (Javed *et al*., 2023). In the current study, risk behavior analysis revealed that 37% of participants reported unprotected sex, 35% shared needles, and 17% had a history of blood transfusions and 11% were MSM. The increasing evidence of sexually transmitted HCV and HBV in HIV-infected MSM are aligned with the previous studied stating the incidence of HCV infection among HIV seropositive MSMs increased (Zheng *et al*., 2021). Sexually acquired infection of HCV has been usually linked with traumatic anal receptive intercourse (Strathdee *et al*., 2021). On the other hand, HBV is most often transmitted by sexual intercourse (both heterosexual and between men), followed by injection drug use (Yang *et al*., 2024). Because HIV and HBV share similar modes of transmission, a large proportion comprising around 90% of the people living with HIV show signs of past HBV infection (Cui *et al*., 2023). However, chronic HBV infection, as indicated by the continued presence of hepatitis B surface antigen (HBsAg), has been found in approximately 5% to 15% of HIV-infected individuals globally (Chen *et al*., 2022).

In our study, significantly lower WBC count or leukopenia was recorded in the co-infected patients compared with the mono-infected group. Leukopenia, defined as a white blood cell count of less than 4,000/μL, and lymphopenia, defined as a lymphocyte count of less than 1,500/μL, are common hematological abnormalities in HIV infection (Haigentz Jr *et al*., 2022). These conditions are associated with an increased risk of infections and are indicative of advanced immune system dysfunction (Obeagu *et al*., 2023). Additionally, the observation of diminished CD4+ T cell counts in co-infected patients is clinically significant. The overall average CD4 count (295.83 cells/μL) and high viral load (mean 1,053,500 copies/mL) is indicative of the substantial immune suppression (Han *et al*., 2021). Moreover, this depletion is aligned with studies suggesting that chronic hepatitis viral infections may impair immune reconstitution under ART. In addition to direct viral killing, HIV-infected CD4+ T cells are targeted for destruction by the immune system. CD8+ cytotoxic T lymphocytes, which recognize and kill infected cells, play a significant role in this process (Kervevan and Chakrabarti, 2021). However, chronic activation and exhaustion of CD8+ T cells impair their ability to control the virus effectively, leading to the persistence of infected cells (Obeagu and Obeagu, 2024). The significant alterations observed in hemoglobin, RBC, and platelet counts indicate that viral hepatitis co-infection amplifies the immunosuppressive and hematopoietic effects of HIV. These alterations align with previous findings suggesting that both HIV and hepatotropic viruses in the disruption of bone marrow function and hematopoietic regulation (Gobran *et al*., 2021). Anemia in HIV infected individuals may be due to different factors specifically the factors influencing bone marrow hematopoietic stem cells, opportunistic infections and ART can also cause anemia in HIV infected patients (Mandania, 2024). HIV-associated thrombocytopenia is often immune mediated, similar to immune thrombocytopenic purpura (ITP). The immune system produces antibodies against platelets, leading to their destruction in the spleen (Hayajenh *et al*., 2021).

Co-infection with HBV and HCV adds another layer of complexity to HIV care. In this study, co-infected patients had significantly worse liver profiles than mono-infected patients. Elevated liver enzyme levels, particularly ALT, AST, and ALP, in the co-infected group point to ongoing hepatic inflammation and potential subclinical fibrosis, possibly due to both viral replication and ART-related hepatotoxicity (Wilkins *et al*., 2013). Our findings further demonstrated that HIV patients co-infected with HBV or HCV exhibit signs of cholestasis, as evidenced by elevated bilirubin levels. The other study showed the same pattern as rise in liver enzymes ALT, AST indicates liver hepatotoxicity greater in co-infected patients receiving ART (Katamba *et al*., 2020). Previous studies have suggested that HIV-infected individuals with chronic HBV infection is typically indicated by persistent hepatitis B surface antigen (HBsAg) positivity. These patients are at increased risk of developing progressive liver complications, including fibrosis, cirrhosis, end-stage liver disease (ESLD), and hepatocellular carcinoma (HCC) (Kechagias *et al*., 2022). In populations without HIV infection, the risk of progression to cirrhosis and HCC among those who are HBsAg positive is significantly associated with factors such as male sex, advancing age, tobacco use, alcohol intake, elevated serum alanine aminotransferase (ALT) levels, and high HBV DNA concentrations (exceeding 10,000 copies/mL) (Hamid *et al*., 2021).

Moreover, a considerable proportion of participants exhibited clinical symptoms indicative of advanced HIV infection. Fever (60%), weight loss (59%), and chronic cough (46%) were common, especially in individuals co-infected with HBV and/or HCV. Additionally, a statistically significant association was observed between chronic diarrhea and co-infection status ($p = 0.036$), that is suggestive of more severe clinical manifestations in co-infected patients. These results align with existing evidence that viral hepatitis co-infections can accelerate HIV disease progression and worsen clinical outcomes (Cheng *et al*., 2021). Therefore, early screening and integrated management of co-infections are therefore crucial for improving patient care and prognosis.

CONCLUSION

Due to shared modes of transmission, coinfection with HBV and/or HCV is common among HIV-infected individuals. The present study has revealed that HIV-positive individuals from the Punjab, Pakistan which were receiving ART and were co-infected with viral hepatitis such as HBV and HCV exhibited significantly worse hematological and biochemical profiles compared to those having HIV mono-infection. Dual Co-infection of both HBV and HCV with HIV was associated with lower CD4 counts, more severe anemia, thrombocytopenia, and elevated liver enzymes, indicating greater immune suppression and liver damage. These findings indicate that co-infections accelerate disease progression and organ damage. Therefore, an early screening, targeted treatment, and integrated care for co-infections are essential to reduce morbidity and improve clinical outcomes in HIV patients under ART in Pakistan.

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IRB approval

IRB approved was obtained from the IRC/BMR, UVAS Lahore (No. 301/IRC/BMR, 07/11/2024).

Ethical statement

All procedures followed institutional ethical guidelines.

Statement of conflict of interest

The authors have declared no conflict of interest regarding the publication of this article.

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