# Effects of HBV And HCV On CD4+ T-Lymphocytes Count and Two Liver Enzymes of HIV-Infected Patients in Orlu, Imo State, Nigeria

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## ABSTRACT

Co-infection of Hepatitis B virus (HBV) and Hepatitis C virus (HCV) are very prevalent and the primary cause for morbidity and mortality among patients with Human immunodeficiency virus (HIV). The aim of the study was to assess the effect of HBV and HCV on CD4+ count and two liver enzymes; Aspartate transaminase (AST) and Alanine transaminase (ALT), among HIV-infected patients. The study was a hospital based cross-sectional study conducted at the anti-retroviral therapy (ART) clinics in Orlu, Imo State, Nigeria. Whole blood samples were collected from 720 patients and serologically analyzed for HBsAg and Anti-HCV using test strips. The samples were also analyzed for the CD4+ count using Cyflow conting system, and two liver enzymes (AST and ALT) using an auto-chemistry analyzer (Cobas C111). Data were entered and analyzed by SPSS version 29 and p-value less than 0.05 was taken as significant. Of the 720 patients, 514 (71.4%) were females and 206 (28.6%) were males. The overall prevalence of HIV/HBV, HIV/HCV, HIV/HBV/HCV, and HIV alone were 332 (46.1%), 82 (11.4%), 105 (14.6%), and 201 (27.9%), respectively. Generally, HIV patients co-infected with HBV and/or HCV had lower mean CD4+ count, AST, and ALT compared to those with HIV alone, but only that of those co-infected with both HBV and HCV were statistically significant ($p < 0.05$). The HIV/HBV/HCV patients have CD4+ count, AST, and ALT of 76.25 cell/µL, 13.47 IU/L, and 12.20 IU/L, respectively compared to 506.60 cell/ µL, 199.81 IU/L, and 65.85 IU/L observed among those having only HIV, respectively. In a unisex comparison, the male patients with HIV/HBV had significantly lower AST and ALT than male patients with HIV only ($p < 0.05$). But, male and female patients with HIV/HBV/HCV had significantly lower AST and ALT compared to the males and females with HIV only ($p < 0.05$), respectively. Across gender, only HIV/HBV/HCV patients had significantly lower CD4+ and AST in males compared to females ($p < 0.05$). There was no statistically significant correlation of CD4, AST and ALT with age in all the groups ($p > 0.05$). In conclusion, HBV and HCV co-infected HIV patients are more likely to have a lower CD4+ counts, liver enzyme levels (AST and ALT) than mono-infected HIV patients, highlighting the detrimental impact of these coinfections on immune functions as well as the crucial role of hormonal factors and health-seeking behaviors on the clinical outcomes of liver enzymes among the co-infected patients.

**Keywords:** Human immunodeficiency virus, Hepatitis B, Hepatitis C, Co-infection, CD4+, Aspartate transaminase, Alanin transaminase, and Immunocompromised**.**

## INTRODUCTION

Hepatitis refers to liver injury characterized by the presence of inflammatory cells in the liver tissue.

The term "hepatitis" is derived from ancient Greek words, "hepar" or "hepato," meaning "liver," and "itis," meaning "inflammation" (Grossman, 2006).

Hepatitis B virus (HBV) is a DNA virus that infects the liver of hominoidae, including humans, resulting in viral hepatitis. Globally, 350-400 million people are chronic carriers of HBV (Hamoud and Mohsen, 2022), of whom 30.4 million are aware of their infection status, and only 6.6 million have received treatment (Cui *et al*., 2023). Transmission occurs through contact with infectious blood or bodily fluids containing blood contaminated with HBV (Alsulaimany, 2023). The HBV infection can manifest as acute (self-limiting) or chronic (long standing), with the majority of individuals having healthy immune systems being able to clear the virus. Symptoms of acute cases include liver inflammation, vomiting, jaundice, and in rare cases, death (Shiffman, 2020). Chronic hepatitis B can progress to cirrhosis and liver cancer, which responds poorly to chemotherapy (Rehermann, 2024). Vaccination effectively prevents HBV infection (Wong *et al*., 2022).

Hepatitis C is a blood-borne infectious disease caused by the Hepatitis C virus (HCV), an RNA virus from the flaviviridae family that significantly affects the liver (Void-Holmes and Cartee, 2024). Approximately 150-200 million people worldwide are infected with HCV, with 3-4 million new cases arise each year (Hamoud and Mohsen, 2022). Transmission occurs through blood-to-blood contact, commonly via unscreened blood transfusions, unsterilized injection equipment, or sexual intercourse, particularly in individuals co-infected with sexually transmitted diseases (STDs) like HIV (Stroffolini and Stroffolini, 2024). The infection can remain asymptomatic for 10-20 years and may advance to chronic hepatitis, culminating in fibrosis, cirrhosis and subsequently, liver failure or other complications of cirrhosis including liver cancer (Garrido and Macedo, 2022) or life threatening splenomegaly, portal hypertensive gastropathy, esophageal and gastric varices (Brzdęk *et al.,* 2024). While no vaccine is currently available for HCV, several candidates are in development, some of which have shown encouraging results (Adugna, 2023).

Human Immunodeficiency Virus (HIV) weakens the body's immune response, making it difficult to fight off infections and illnesses (Prabhu and van Wagoner, 2023). A robust cell-mediated immune response involving both CD4+ and CD8+ cells is crucial for the managing and control of HCV and HBV (Saraceni and Birk, 2021). In HIV-infected individuals, the compromised immune response increases the likelihood of HBV and HCV replication, making it difficult for the body to clear these viruses (Shahriar *et al*., 2022). Co-infection refers to the presence of multiple infectious agents. Thus, co-infection refers to infection with two or more different disease -causing organisms (Ramlal *et al.,* 2023).

HIV co-infection with HBV and/or HCV is associated with increased risk of liver-related morbidity and mortality (Gad and Elagrody, 2020). But chronic hepatitis is a risk factor for drug or immune mediated liver injury during HIV treatment (Navarro, 2022). With early diagnosis of HIV infection and effective antiretroviral treatment leading to improved survival of patients with HIV infection, hepatitis B- and C-related liver disease is emerging as a significant health problem in this group of patients (Osagiede *et al*., 2020).

A better understanding of these issues is essential in the South-East region of Nigeria to guide the prevention and management strategies. Therefore, there is a need to determine the impact of co-infection or triple infection on baseline serum alanine transaminase (ALT), aspartate transaminase (AST), and CD4+ T-lymphocyte counts in HIV-infected patients in Orlu, Imo State, South-East Nigeria.

## MATERIALS AND METHODS

### Materials

### Study Area

This study was carried out at Orlu, Imo State, South-Eastern Nigeria, and is located within latitude 5º42′N and 5º52′N, and longitude 6º56′E and 7º07′E. Imo State shares boundary in the North with Anambra State, in the

South and West with Rivers State and in the East with Abia State. Orlu is one of the three senatorial zones in Imo State comprising of 12 Local Government Areas in the State. While exact and current population figures can vary, as of the last census conducted in 2006, Imo State has a standardized population of 3,934,899 million based on the estimated census figure of 2006 (National Bruea of Statistics, 2006). The Imo State University Teaching Hospital (IMSUTH) and International Market which are both located within Orlu metropolis attracted many banks and leisure houses to the area. Thus, majority of the inhabitants are students, civil servants, bankers, and business men and women. Hence, the standard of living is average, although few of the populace appears to be living below poverty level.

**Study Population**

The study population comprises of 720 HIV-positive patients who were attending two major Hospitals' Counseling and testing (HCT) Units in Orlu metropolis namely: Imo State University Teaching Hospital and St. Damian's Hospital, Okporo.

**Study Design**

The study was a cross-sectional study.

**Test Samples**

A total of 720 whole blood samples were collected aseptically by Medical Laboratory Scientists and were analyzed with standard methods.

**Test Reagents**

The test reagents used for test analysis were: third generation Enzyme Immunosorbent Assay (ELISA) kit, HBsAg and HCV rapid kits, Determine rapid kit, Unigold rapid kit, Stat-pak rapid kit, Partec cleaning solution, sheath fluid, decontamination fluid, count check beads, CD4abs.antibody, No Lyse buffer, 2, 4 dinitrophenylhydrazine, 0.4N sodium hydroxide, and RANDOX kit for Aspartate transaminase and Alanine transaminase.

**Data Collection**

Informed consent was obtained from all participating patients involved in the study, and their age and sex were recorded.

**Methods**

**Ethical Approval**

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of Imo State University Teaching Hospital, Orlu.

**Selection of Respondents**

The participating patients were randomly selected based on confirmed HIV-positive patients who were attending HIV-clinic on Tuesdays and Thursdays within the period of study, from March to September, 2023.

The followings were the inclusion and exclusion criteria employed in the selection of the participating patients:

1. Patients should be adults aged 18 years and older.
2. Patients can be of any gender.

3. Patients must be HIV-positive.
4. Patients must provide informed consent to participate in the study.
5. Patients should not be pregnant or breastfeeding.
6. Patients should not have any other significant medical condition(s) that could affect the liver enzymes or CD4+ count.

**Collection of Samples**

Whole blood samples were collected from the confirmed HIV-positive patients using 5 mL vacutainer EDTA and plain containers. The sample in EDTA containers were used in the analysis of CD4+ count, while those in plain containers were separated and the serum used in HBsAg, HCV, ALT and AST analysis.

**Processing of Samples**

The 720 participating patients were reconfirmed as HIV-positive through rescreening for HIV antibodies using the approved National Serial Algorithm. The initial screening was conducted with Determine rapid kits, and if positive, confirmation was done using Unigold rapid kits. Stat-Pak rapid kits were employed in cases of discordant results (Okonko and Shaibu, 2023).

The collected blood samples were analyzed in the IMSUTH-Orlu Reference Laboratory. The whole blood samples from 720 confirmed HIV-positive patients were separated and the serum assayed for the presence of antibodies to HBV (HBsAg) and HCV (anti-HCV) using screening test strips. Further confirmation was carried out by qualitative ELISA system (microplate reader and washer-Accurex USA) using third generation ELISA kit (Nwangwu et al., 2022).

T-lymphocyte subsets CD4+ count and liver enzyme (AST and ALT) level were obtained using Cyflow counting system (Partec Cy-flow; SL-3 Green) and an auto-chemistry analyzer (Cobas C111), respectively, in strict adherance to the manufacturer's protocols during each assay (Ajugwo et al., 2020; Ezeugwunne et al., 2021).

The HBV-Full and HCV negative patients were used as controls to assess the impact of HBV and HCV on the CD4+ count and liver enzymes (AST and ALT) of HIV-positive patients.

**Data Analysis**

Data analysis was done using Statistical Package for Social Sciences (SPSS) version 29 (Chicago, USA). The Chi-Square test, Pearson Correlation test, and analysis of variance (ANOVA) were used to assess the significance of the difference among the groups. A value of $p \leq 0.05$ was considered statistically significant.

## RESULTS

**Figure 1** shows the sex distribution of the co-infected and mono-infected HIV Patients. An overall of 720 participants were recruited for this study, of which 514 (71.4%) were females and 206 (28.6%) were males. Based on coinfection and mono-infection, 332 (46.1%) had HIV/HBV, 82 (11.4%) had HIV/HCV, 105 (14.6%) had HIV/HBV/HCV, and 201 (27.9%) had HIV only. Based on sex, female participants had the highest prevalence in all the categories at 81.0%, 61.0%, 69.5%, 60.7% in HIV/HBV, HIV/HCV, HIV/HBV/HCV, and HIV only groups, respectively compared to the male counterpart at 19.0%, 39.0%, 30.5%, and 39.9% prevalence in HIV/HBV, HIV/HCV, HIV/HBV/HCV, and HIV only groups, respectively.

**Table 1** shows mean age of the co-infected and mono-infected HIV patients. It showed that there was no statistically significant difference in the mean age across the groups ($p > 0.05$).

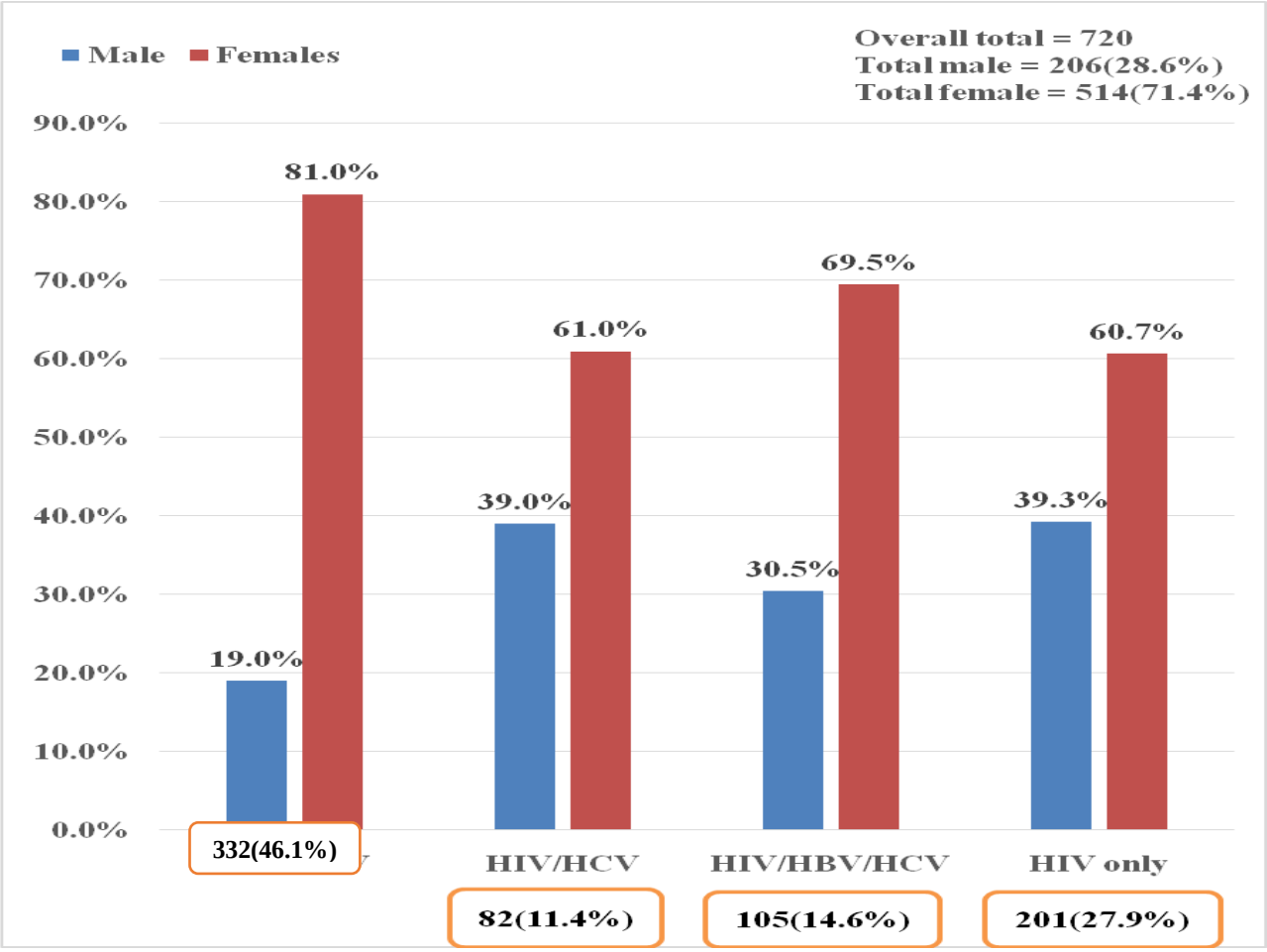


**Figure 1: Sex Distribution of the Co-infected and Mono-infected HIV Patients**

Table 1: Mean Age of the Co-Infected and Mono-infected HIV Patients N = 720

| Group | No of participants | Mean±SD | f-value | p-value |
|---|---|---|---|---|
| **HIV/HBV** | 332 | 35.40±14.54 | 0.261 | 0.853 |
| **HIV/HCV** | 82 | 29.00±17.76 | | |
| **HIV/HBV/HCV** | 105 | 35.25±20.17 | | |
| **HIV Only** | 201 | 33.44±15.04 | | |

**Note:** f-value = One-way ANOVA; p-value = Significant level

**Table 2** shows the effect of HBV and HCV co-infection on CD4+ count and two liver enzymes of HIV-infected patients. The table showed statistically significant different in the CD4+ count ($p < 0.05$), AST ($p < 0.001$), and ALT ($p < 0.001$) across the groups. Although the CD4+ count, AST and ALT of the coinfected groups (HIV/HBV, HIV/HCV, and HIV/HBV/HCV) were lower compared to that of the mono-infected group (HIV only), a Dunnett post-hoc test that compares every mean to a control mean revealed only the HIV/HBV/HCV group have statistically significant lower CD4+ count ($p = 0.011$), AST ($p = <0.001$) and ALT ($p = <0.001$) as compared to that of the mono-infected group (HIV only).

Table 2: Effect of HBV and HCV Coinfection on CD4+ Count and Two Liver Enzymes of HIV-Infected Patients N = 720

| Group | N | Mean±SD | | |
|---|---|---|---|---|
| | | CD4+ count (Cells/µL) | AST (IU/L) | ALT (IU/L) |
| **HIV/HBV** | 332 | 368.00±278.09 | 149.22±73.07 | 51.43±22.70 |
| **HIV/HCV** | 82 | 243.80±121.30 | 193.60±18.74 | 71.94±4.66 |
| **HIV/HBV/HCV** | 105 | **76.25±52.44*** | **13.47±2.69*** | **12.20±9.00*** |
| **HIV Only** | 201 | 506.60±232.55 | 199.81±21.29 | 65.85±4.36 |
| **f-value** | | 3.697 | 13.474 | 13.056 |
| **p-value** | | **0.022** | **<0.001** | **<0.001** |

**Note:** f-value = One-way ANOVA; p-value = Significant level, N = Number of participants "*" = Significant when compared to the mono-infected patients (HIV only).

**Table 3** shows the effect of HBV and HCV coinfection on CD4+ count and two liver enzymes of HIV-infected patients based on male gender. Statistically significant difference was observed only in the AST ($p < 0.01$), and ALT ($p < 0.01$) across the groups, which was further analyzed using a Dunnett post-hoc test that compares every mean to a control mean and it revealed the HIV/HBV and HIV/HBV/HCV as the groups statistically significantly lower in AST ($p = 0.034$; $p = 0.006$, respectively) and ALT ($p = 0.049$; $p = 0.006$, respectively) as compared to that of the mono-infected group (HIV only).

Table 3: Effect of HBV and HCV Co-Infection on CD4+ Count and Two Liver Enzymes of HIV-Infected Patients Based on Male Gender N = 206

| Group | N | Mean±SD | | |
|---|---|---|---|---|
| | | CD4+ count (Cells/µL) | AST (IU/L) | ALT (IU/L) |
| **HIV/HBV** | 63 | 334.75±289.52 | **87.49±91.49*** | **33.26±29.08*** |
| **HIV/HCV** | 32 | 180.50±152.03 | 203.39±12.95 | 71.30±3.54 |
| **HIV/HBV/HCV** | 32 | 31.50±10.61 | **11.21±1.12*** | **5.40±4.95*** |

| | | | | |
|---|---|---|---|---|
| **HIV Only** | 79 | 434.20±293.89 | 198.25±6.22 | 66.39±4.64 |
| **f-value** | | 1.294 | 8.141 | 8.200 |
| **p-value** | | 0.335 | **0.006** | **0.006** |

**Note:** f-value = One-way ANOVA; p-value = Significant level, N = Number of participants "*" = Significant when compared to the mono-infected patients (HIV only).

**Table 4** shows the effect of HBV and HCV coinfection on CD4+ count and two liver enzymes of HIV-infected patients based on female gender. Statistically significant difference was observed only in the AST ($p < 0.01$), and ALT ($p < 0.001$) across the groups, which was further analyzed using a Dunnett post-hoc test that compares every mean to a control mean and it revealed the HIV/HBV/HCV as the only group statistically significantly lower in AST ($p = 0.002$) and ALT ($p = <0.001$) as compared to that of the mono-infected group (HIV only).

Table 4: Effect of HBV and HCV Co-Infection on CD4+ Count and Two Liver Enzymes of HIV-Infected Patients Based on Female Gender N = 514

| **Group** | **N** | **Mean±SD** | | |
|---|---|---|---|---|
| | | **CD4+ count (Cells/µL)** | **AST (IU/L)** | **ALT (IU/L)** |
| **HIV/HBV** | 269 | 380.09±287.26 | 171.67±53.72 | 58.04±16.95 |
| **HIV/HCV** | 50 | 286.00±105.79 | 187.07±21.42 | 72.37±6.04 |
| **HIV/HBV/HCV** | 73 | 121.00±11.31 | **15.72±0.40*** | **18.99±5.81*** |
| **HIV Only** | 122 | 579.00±149.01 | 579.00±149.01 | 65.31±4.53 |
| **f-value** | | 2.159 | 7.270 | 8.936 |
| **p-value** | | 0.130 | **0.002** | **<0.001** |

**Note:** f-value = One-way ANOVA; p-value = Significant level, N = Number of participants "*" = Significant when compared to the mono-infected patients (HIV only).

**Table 5** shows the CD4+ count and two liver enzymes cross gender of co-infected and mono- infected HIV patients. The table showed that only HIV/HBV/HCV group have a statistically significant difference across gender. This difference was observed in the CD4+ count ($p < 0.05$) and ALT ($p < 0.05$), with both parameters higher in the females compared to the male.

**Table 6** shows the correlation of CD4+ count and two liver enzymes with age of co-infected and mono-infected HIV patients. The Pearson correlation showed that the parameters – CD4+ count, AST and ALT

have no statistically significant correlation with age in both coinfected and mono-infected HIV patients (HIV/HBV, HIV/HCV, HIV/HBV/HCV and HIV only).

Table 5: The CD4+ Count and Two Liver Enzymes Cross Gender of Co-Infected and Mono-Infected HIV Patients

| Group | Parameter | Male | Female | t-value | p-value |
|---|---|---|---|---|---|
| **HIV/HBV** | n | 63 | 269 | | |
| | CD4+ (Cells/µL) | 334.75±289.52 | 380.09±287.26 | -0.270 | 0.792 |
| | AST (IU/L) | 87.49±91.49 | 171.67±53.72 | -1.735 | 0.162 |
| | ALT (IU/L) | 33.26±29.08 | 58.04±16.95 | -1.607 | 0.188 |
| **HIV/HCV** | n | 32 | 50 | | |
| | CD4+ (Cells/µL) | 180.50±152.03 | 286.00±105.79 | -0.938 | 0.417 |
| | AST (IU/L) | 203.39±12.95 | 187.07±21.42 | 0.940 | 0.417 |
| | ALT (IU/L) | 71.30±3.54 | 72.37±6.04 | -0.220 | 0.840 |
| **HIV/HBV/HCV** | n | 32 | 73 | | |
| | CD4+ (Cells/µL) | 31.50±10.61 | 121.00±11.31 | -8.162 | 0.015 |
| | AST (IU/L) | 11.21±1.12 | 15.72±0.40 | -5.381 | 0.033 |
| | ALT (IU/L) | 5.40±4.95 | 18.99±5.81 | -2.517 | 0.128 |
| **HIV only** | n | 79 | 122 | | |
| | CD4+ (Cells/µL) | 434.20±293.89 | 579.00±149.01 | -0.983 | 0.355 |
| | AST (IU/L) | 66.39±4.64 | 65.31±4.53 | 0.371 | 0.720 |
| | ALT (IU/L) | 198.25±6.22 | 201.36±31.23 | -0.218 | 0.833 |

**Note:** t-value = Independent sample test; p-value = Significant level, n = Number of participants

Table 6: The Correlation of CD4 Count and Two Liver Enzymes with Age of the Co-Infected and Mono-Infected HIV Patients

| Group | Parameter | r-value | p-value |
|---|---|---|---|
| **HIV/HBV** | CD4 (Cells/µL) | -0.502 | 0.057 |
| | AST (IU/L) | 0.250 | 0.369 |
| | ALT (IU/L) | 0.257 | 0.355 |
| **HIV/HCV** | CD4 (Cells/µL) | -0.142 | 0.819 |
| | AST (IU/L) | 0.187 | 0.763 |

| | | | |
|---|---|---|---|
| | ALT (IU/L) | 0.151 | 0.808 |
| **HIV/HBV/HCV** | CD4 (Cells/µL) | -0.719 | 0.302 |
| | AST (IU/L) | -0.941 | 0.059 |
| | ALT (IU/L) | -0.848 | 0.152 |
| **HIV only** | CD4 (Cells/µL) | -0.472 | 0.169 |
| | AST (IU/L) | -0.147 | 0.686 |
| | ALT (IU/L) | -0.135 | 0.711 |

**Note:** r-value = Pearson's Correlation Test; p-value = Significant level.

## DISCUSSION

Hepatitis B and C related liver diseases are emerging as significant health problems in patients living with the HIV, even with early diagnosis of HIV infection and effective anti-retroviral treatment.

Patients with HIV who were co-infected with hepatitis B virus (HBV) and/or hepatitis C virus (HCV) exhibited lower CD4+ counts compared to those with HIV alone, but only that of those co-infected with both HBV and HCV showed a statistically significant decrease in CD4+ count. This is primarily due to the increased immune system compromise caused by the co-infection, where the presence of HBV and HCV can further weaken the immune response, thereby exacerbating the effects of HIV (Shahriar et al., 2022), by making it less likely for those with co-infection to experience a CD4+ count increase of at least 50cells/$mm^3$ after starting HIV treatment (Nahar et al., 2021). The significant low CD4+ count among those co-infection with HBV and HCV might be due to the robust combined impact of both HBV and HCV on the body's immune system.

These observations correspond with the work of Bazmjoo et al. (2023) who reported a significant decline in CD4+ counts ($p < 0.05$) among HIV patients co-infected with HBV and HCV at health centers of Jahrom and Fasa cities in Iran. Suman (2020) also reported a lower mean CD4+ count among those positive for both HBsAg and anti-HCV than those who were negative for both ($p < 0.05$). These researchers did not provide specific CD4+ count rather the graphically showed the decline in the CD4+ count among the HIV patients co-infected with HBV and HCV.

Although in a unisex comparison, CD4+ count was lower among HIV patients co-infected with HBV and/or HCV but was not statistically significant. This may be attributed to the wide difference in sample size of each group and the complex interactions between HIV, HBV, and HCV as well as the confounding variables and biological variations (Ellwanger et al., 2020) which in the study populations may hinder a statistically significant result. But across gender, only HIV patients co-infected with both HBV and HCV exhibited significantly lower CD4+ count in males compared to females. This maybe as a result of biological differences as males typically have a more robust inflammatory response and higher viral loads in the context of infections (Moran et al., 2022), which can contribute to quicker CD4+ cell depletion. Secondly, hormonal factors may influence immune response, as estrogen in females has been shown to have a protective effect on immune function, potentially leading to better CD4+ cell preservation (Orzołek et al., 2022).

Patients with HIV who were co-infected with HBV and/or HCV exhibited lower liver enzymes (AST and ALT) compared to those with HIV alone but only that of those co-infected with both HBV and HCV showed a statistically significant decrease in liver enzymes concentration. In contrast, a study by Gad and Elagrody (2020) found that the mean levels of ALT and AST were highest among patients co-infected with HBV (102.0 U/L and 95.6 U/L, respectively), followed by those co-infected with both HBV and HCV (93.2 U/L and 89.2 U/L, respectively), and lowest among those co-infected with HCV (36.4 U/L and 34.3 U/L, respectively). The study also indicated that ALT and AST levels were significantly higher in patients co-infected with HBV and those co-infected with both HBV and HCV compared to mono-infected HIV patients (31.9 U/L and 29.6 U/L,

respectively) (p = 0.001). The variation between the studies maybe attributed to the difference in ART treatment and the patients' compliance to the ART treatment.

Patients on effective ART may experience improvements in immune functions and reduction in liver injury, but the specific ART regimens can have varying impacts on liver functions (Navarro, 2022). Direct-acting antiviral (DAA) therapies for HCV are particularly effective in reducing liver inflammation and potentially leading to lower enzyme levels (Ferreira et al., 2024). Conversely, treatment for HBV might not have the same effect and can result in fluctuating liver enzymes production based on disease activity (Iannacone and Guidotti, 2022). Furthermore, differences in adherence to treatment regimens can affect liver health outcomes, with lower adherence rates potentially leading to higher liver enzyme levels and greater liver damage (Jacquelet et al., 2021).

In a unisex comparison, only in the male HIV patients co-infected with HBV were having AST and ALT significantly lower than male with HIV only. Varying health-seeking behaviors and various lifestyle choices can influence liver health outcomes (Jafree et al., 2023), this behavioral characteristic may be pronounced in the male compared to the females leading to the significantly lower AST and ALT in males HIV patients co-infected with HBV when compared to males with HIV alone. Also, male and female HIV patients co-infected with both HBV and HCV have significantly lower AST and ALT than male and females with HIV only, respectively. This shows that gender has a strong influence on the level of AST and ALT among HIV patients co-infected with both HBV and HCV. The significantly lower level of AST and ALT in both genders may be may be attributed to the protective effects of sex hormones. While this may be commonly considered in the context of females (Orzołek et al., 2022), hormonal interactions in males could influence liver health in ways that reduce liver enzyme levels in the presence of both HBV and HCV (Sayaf et al., 2022).

Across gender, HIV patients co-infected with both HBV and HCV exhibit significantly lower AST count in males compared to females. Generally, males benefit from the anti-inflammatory effects of testosterone, which can result in lower liver injury markers (Khaled et al., 2023). Similarly, female estrogen can provide protective benefits against certain liver diseases (Orzołek et al., 2022). In this situation, the male hormone may have a stronger anti-inflammatory effect than the female hormone.

However, this study also analyzed the correlation of HIV co-infected with HBV and/or HCV with age, but there was no statistically significant correlation of CD4+, AST and ALT with age in both HIV patients co-infected HBV and/or HCV and those with HIV only. The presentation of liver disease and immune status can vary widely among individuals with HIV, HBV, and HCV. Factors other than age, such as drug use, alcohol consumption, coexisting conditions, and genetic predispositions, may have a more substantial impact on liver function and immune response than age alone (Åberg et al., 2023; Daly, 2023; Yang et al., 2023).

## CONCLUSION

The findings of the study indicate that HIV patients co-infected with HBV and/or HCV demonstrate lower CD4+ counts, and liver enzyme levels (AST and ALT) compared to those with HIV alone, highlighting the detrimental impact of these co-infections on immune functions. Notably, the combined effect of HBV and HCV appears to exacerbate immune compromise, further complicating treatment outcomes. The varying impacts of ART and differences in treatment adherence underscore the complexity of managing these coexisting infections. The observed gender differences in CD4+ counts and liver enzyme levels suggest that hormonal factors and health-seeking behaviors play crucial roles in the clinical outcomes of coinfected patients. Despite the lack of significant correlations between age, this study highlights the multi-factorial nature of liver disease and immune status in the context of HIV patients co-infected with HBV and/or HCV, emphasizing the need for tailored management strategies that consider individual patient characteristics and coexisting conditions. Efforts at addressing these factors are essential for improving patient outcomes in this vulnerable population that is immunocompromised.

### Limitation(s)

Like other cross-sectional studies, authors can only describe associations or significant difference between the

variables, and may not determine a etiology or casualty. The study cohort might be subject to selection bias, affecting the representativeness of the population being studied, as the participants were recruited from specific healthcare facilities. Variability in the sensitivity and specificity of the diagnostic tests used could affect the accuracy of the results. Other factors influencing CD4+ counts or liver function tests (such as medication use, or lifestyle factors) may not have been adequately controlled. The findings may not be generalizable beyond Orlu, Imo State, Nigeria, due to regional differences in health care access, population demographics, and prevalence of infections.

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**Conflicts of Interest**

The authors declare that there is no conflict of interest.

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