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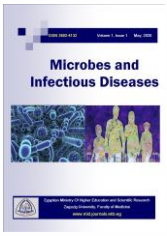

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Original article

Impact of hepatitis B virus infection on CD4 T-cell count and ALT in HBV infected individuals: Initial insight from an underprivileged setting

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ABSTRACT

Background: This study aims to investigate the relationship between CD4+ T-cell counts, alanine aminotransferase (ALT) levels and HBV-DNA in acute and chronic HBV patients across varying serum ALT levels. **Methods**: In this cross-sectional study, 200 participants were enrolled, with an equal distribution of acute and chronic hepatitis B virus (HBV)-positive cases. HBsAg, absolute CD4+ T-cell count, HBsAg, ALT and HBV-DNA were measured using standard methods. Cases were grouped into acute or chronic HBV subjects using qualitative laboratory tests. **Results**: The study found a high prevalence of abnormal ALT levels in HBV-infected cases, with 100% of acute cases and 75% of chronic cases showing increased ALT levels. The median levels of ALT, CD4+ T-cell count, and HBV viral load were compared between acute and chronic HBV-positive cases. The results showed that ALT levels correlated positively with HBV-DNA and CD4 T-cell count, while HBV viral load correlated positively with CD4 T-cell count in acute infected cases only. Interestingly, increasing viral hepatitis B load was associated with a decreased CD4+ T-cell count and increased ALT levels. **Conclusion**: Our findings shed light on the dynamic nature of HBV infection, highlighting differences between acute and chronic cases in terms of liver enzyme levels, immune responses, and viral replication. Abnormal ALT levels may serve as an important clinical indicator of active HBV replication and its potential impact on the immune system. Monitoring these parameters aids in assessing and managing HBV-infected individuals, particularly those with abnormal liver enzyme levels.

Introduction

Hepatitis B is a viral infection that affects the liver and can cause inflammation, liver cirrhosis, and hepatocellular carcinoma (HCC) [1]. It is caused by the hepatitis B virus (HBV), which is transmitted through blood, semen, or other body fluids from an infected person to an uninfected person [2]. The virus can be spread through unprotected sex, sharing needles, accidental needle sticks, and from mother to child during childbirth. Symptoms of hepatitis B include fever, skin rash, and polyarteritis, and can range from an asymptomatic carrier state to chronic hepatitis, liver cirrhosis, and HCC [1, 3]. Chronic hepatitis B has a variable and dynamic course, and early during infection, HBeAg, HBsAg, and HBV DNA are usually present in high titers [4]. Although most people will recover from acute illness, some people with chronic hepatitis B will develop

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progressive liver disease and complications like cirrhosis and hepatocellular carcinoma (liver cancer) [1]. According to the World Health Organization, approximately 296 million people are currently living with hepatitis B, and the burden disproportionately affects sub-Saharan Africa and East Asia [5,6].

CD4 T lymphocytes play an essential role in coordinating immune responses, and studies indicate that they are involved in both controlling and contributing to the pathogenesis of HBV infection [7]. The changes that occur in CD4 T lymphocytes during HBV infection are associated with disease progression, leading to a compromised cellular immune response [8,9,10]. As a result, the overall immune function of the body is weakened, making it challenging to clear the virus and leading to prolonged disease duration without resolution [11]. During chronic HBV infections, virus-specific T-cells appear deeply exhausted, and both CD8 and CD4 T cells up-regulate co-inhibitory receptors, which can inhibit T cell function upon cross-linking of their corresponding ligands [12,13,14]. CD4 T cells are severely dysfunctional in chronic HBV infection as a result of several inhibitory mechanisms that are simultaneously active within the chronically inflamed liver [15,16,17,18]. In HBV infection, a decrease in CD4+ T-cell count has been linked to weakened immune response and elevated viral replication. Busca and Kumar [17] conducted a study investigating the association between CD4+ T-cell count and HBV-DNA levels in individuals with chronic HBV. The findings indicated that individuals with lower CD4+ T-cell counts exhibited higher HBV-DNA levels, indicating a negative relationship between CD4+ T-cell count and HBV replication [16,17,18,19].

Alanine aminotransferase (ALT) is an enzyme produced in response to cell damage or death. Functioning as a biomarker, its serum levels can aid in diagnosing and predicting the course of diseases and injuries resulting from viral hepatitis affecting the liver [20]. In cases of acute HBV infection, aminotransferases, particularly ALT, typically show a notable surge, sometimes exceeding a 100-fold increase. On the other hand, chronic HBV infection commonly manifests with ALT levels either within normal range or elevated, reaching up to 200 IU/L in approximately 90% of patients [21,22].

The intricate relationship between HBV-DNA levels, CD4+ T-cell counts, and ALT levels remains a subject of exploration. Existing studies suggest a lack of significant correlation between HBV-DNA levels and CD4+ T-cell counts in chronic HBV patients with normal ALT levels [22,23]. However, there is limited literature available to examine the impact of hepatitis B virus on CD4 T lymphocytes in the presence of varying ALT levels from an underprivileged setting. Hence, the present study aimed to investigate the interplay between CD4+ T-cell counts, alanine aminotransferase (ALT) levels, and HBV-DNA, emphasizing their potential impact on both chronic and acute HBV patients across various serum ALT levels in an underprivileged setting [22,23].

Materials and Methods

Study design

A cross-sectional study was conducted to investigate the impact of HBV infection on CD4 T lymphocytes in different serum ALT strata. Participants with chronic and acute HBV infection were recruited and stratified into three groups based on their serum ALT levels: low, moderate, and high. This study was conducted between August 2022 and January 2023 at the antiretroviral therapy (ART) Laboratory of the Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, Nigeria.

Ethics approval

The study protocol was approved by the hospital research ethics committee of ABUTH, Zaria, Nigeria (ethical approval number: ABUTHZ/HREC/B23/2019). Informed consent was obtained from all the participants in accordance with Helsinki Declaration of 1975, as revised in 2000; voluntariness and strict confidentiality were maintained throughout the study.

Study participants

In this study, a convenience sampling method was used to select a group of 200 adults ranging from 18 to 80 years old. The participants consisted of 100 individuals diagnosed with acute HBV infection and another 100 individuals diagnosed with chronic HBV infection.

Sociodemographic and clinical data

A structured questionnaire and a thorough examination of the patient's clinical records were utilized to gather pertinent sociodemographic characteristics and clinical information.

Specimens collection and laboratory analysis

Ten milliliters (10 mL) of venous blood were collected from each participant into EDTA and plain bottles using aseptic techniques. All blood samples collected in the EDTA tubes were immediately analyzed for $CD4^+$ T cell count. The plasma harvested from EDTA tubes was used for HBV-DNA assays. HBV-DNA samples that could not be processed immediately were stored at -800 °C until analysis. The sera harvested from the plain tubes were used for serology and ALT assay.

Detection of HBsAg by ELISA

The ELISA technique, as described by Chang et al. [10], was employed to examine all specimens for the presence of hepatitis B surface antigen. The testing procedure adhered strictly to the instructions provided by Fortress Diagnostics, who supplied the 4th generation ELISA kit from Ireland, UK.

HBcAb-total and HBcAb-*IgM* qualitative test for acute and chronic HBV infection

Subjects infected with HBV (HBsAg positive) were examined for HBcAb-total, and if positive, they were further tested for HBcAb-IgM, an indicator of acute HBV infection. Those with a positive result for HBcAb-IgM were categorized as having an acute infection, while those with a negative result were classified as having a chronic infection. The Advance Quality TM One Step HBcAb-total and HBcAb-IgM test kits from InTec Products China were employed for these assessments.

Flow cytometry analysis for absolute $CD4^+$ T cells count

The CD4+ T-cell count was examined by utilizing the Cyflow counter machine (Partec, Germany), which operates on the principle of flow cytometry [11]. All testing procedures were performed in accordance with the guidelines provided by the manufacturer.

Measurement of alanine amino transferase (ALT)

The measurement of alanine aminotransferase was carried out using the Reiman and Frankel method [12] with Randox reagents and Stax Fax 1904 Spectrophotometer. ALT levels were determined by monitoring the concentration of pyruvate hydrazone formed with 2, 4- dinitrophenylhydrazine. The guidelines and protocols provided by the kit manufacturer were strictly adhered to during the process.

Hepatitis B virus (HBV) DNA detection and quantification by Real-Time PCR

The HBV viral load was assessed utilizing the automated COBAS® Ampliprep/COBAS® Taqman® HBV DNA Test, version 2 (Roche Diagnostics, USA), with a detection range of 20–170,000,000 IU/mL. The sample preparations and PCR amplification followed Chang et al. [10] method, while strictly adhering to the manufacturer's instructions to ensure precise laboratory results.

Definitions

The established thresholds for normal ALT levels were set at 30 U/L or lower for men and 19 U/L or lower for women. High-level replication of HBV was indicated by evidence of HBV DNA exceeding 20,000 IU/mL. Poor immunity was defined as having a CD4 T-cell count below 200 cells/μL [11].

Statistical analysis

1. Data obtained were presented in tables as percentages and median (interquartile range, IQR) or mean ±standard deviation. The Shapiro-Wilks test was employed to check the normal distribution of quantitative variables. According to variable distribution, comparison among groups was analysed using t-test (and non-parametric test) or a non-parametric (Mann-Whitney test). Spearman correlation coefficient (r) was used to find an association between all variables due to its robustness to skewed data. Graphpad prism software (v 6) and "was used for all data analysis. The significance level was set at $P \leq 0.05$

Results

Table 1 outlines the demographics of 200 participants split equally between acute and chronic HBV infection groups. Ages ranged from 18 to 80 with their mean ages (38.7 ± 14.94 and 38.85 ± 14.92 years for acute and chronic HBV–positive cases respectively).

Gender distribution in acute HBV was 44% female, 56% male; Chronic had 25% female, 56% male. The table also details clinical characteristics: all acute cases showed elevated ALT levels, 75% in chronic cases; low CD4+ T-cell count was 51% for acute, 52% for chronic cases. Table 1 further explores HBV manifestations based on liver

enzymes, CD4+ T-cell count, and HBV DNA levels among the groups.

Assessment of alanine aminotransferase, CD4+ T-cell count, and viral load among the study participants

Liver enzyme (ALT) levels varied significantly among groups: Acute HBV cases displayed the highest median ALT (61.0, IQR: 48.8–72.0) U/L, while chronic cases had the lowest (44.00, IQR: 30.25–59.50) U/L, a statistically notable difference ($p < 0.0001$, **Figure 1A**). CD4+ T-cell counts were highest in acute HBV (194.5, IQR: 142.0-308.0) cells/µL and significantly lower in chronic cases (198, IQR: 172.5-218.0) cells/µL ($p < 0.0001$, **Figure 1B**). Conversely, chronic HBV displayed substantially higher HBV-DNA levels (2.960, IQR: 2.420-5.960) Log10(IU/mL) compared to acute cases (2.330, IQR: 1.790-2.914) Log10(IU/mL) ($p < 0.0001$, **Figure 1C**).

Correlation analysis between alanine aminotransferase, CD4 T-cell counts and HBV-DNA among the study group

The study unveils correlations among alanine aminotransferase (ALT), CD4+ T-cell count, and hepatitis B viral load in chronic and acute HBV-infected individuals. In chronic cases, a weak negative correlation exists between ALT and CD4+ T-cell count ($r = -0.1505$), though statistically insignificant ($p > 0.05$). Conversely, acute cases show a moderate positive correlation between CD4+ T-cell count and HBV-DNA levels ($r = 0.2331$), significant at $p < 0.05$, suggesting higher CD4+ T-cell counts correspond to increased HBV-DNA levels. In chronic infections, the correlation between viral load and CD4+ T-cell count is very weak and insignificant ($r = 0.08759$, $p > 0.05$). However, in acute infections, a moderate positive correlation exists, indicating rising CD4+ T-cell counts align with increased viral load ($r = 0.2757$, $p < 0.05$). Moreover, a moderate positive correlation between ALT and viral load is significant in acute cases ($r = 0.2631$, $p < 0.05$), implying elevated ALT levels correspond to higher viral loads. In chronic cases, a weak positive ALT-viral load correlation lacks statistical significance ($r = 0.1640$, $p > 0.05$). Overall, the relationships between ALT, CD4+ T-cell count, and viral load vary distinctly in chronic versus acute HBV infections, underscoring the intricate dynamics within hepatitis B virus infection **(Figure 2).**

Table 1. Demographic and clinical characteristics of acute and chronic HBV infection in the study participants

Variables	Acute HBV+	Chronic HBV+	P-value
Age (years), Mean±SD	38.7±14.94	38.85±14.92	N.S
Gender, n (%)			
Female	44 (44)	25 (25)	<0.0001
Male	56 (56)	56 (56)	
CD4+ T cell (count/µL)			
<200	51 (51)	52 (52)	<0.0001
>200	49 (49)	48 (48)	
HBV DNA IU/mL			
<20,000	94 (94)	67 (67)	<0.0001
>20,000	6 (6)	33 (33)	
ALT (U/L)			
Normal	0	25 (25)	<0.0001
Abnormal	100 (100)	75 (75)	

N.S: not significant

Figure 1. Assessment of ALT, CD4 T-cell counts and HBV-DNA among acute and chronic HBV infected study participants. Dots represent each participant along the predefine levels of ALT, CD4 T-cell counts and HBV-DNA. A represents alanine transaminase (ALT) levels among acute and chronic HBV infected study participants; B represents CD4 T-cell count levels among acute and chronic HBV infected study participants and C represents viral load of acute and chronic HBV infected participants; Mann-whitney test (P≤ 0.05).

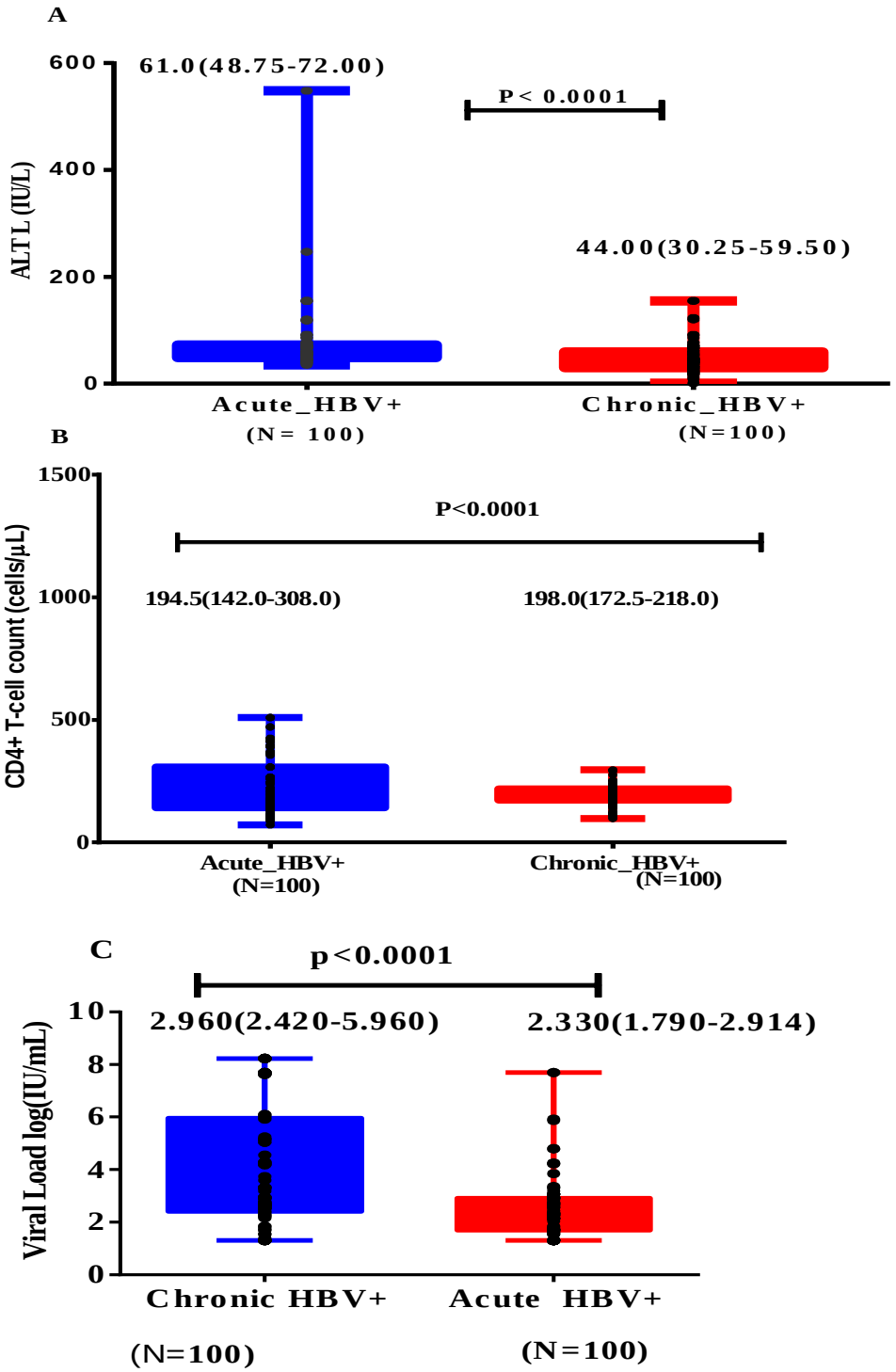
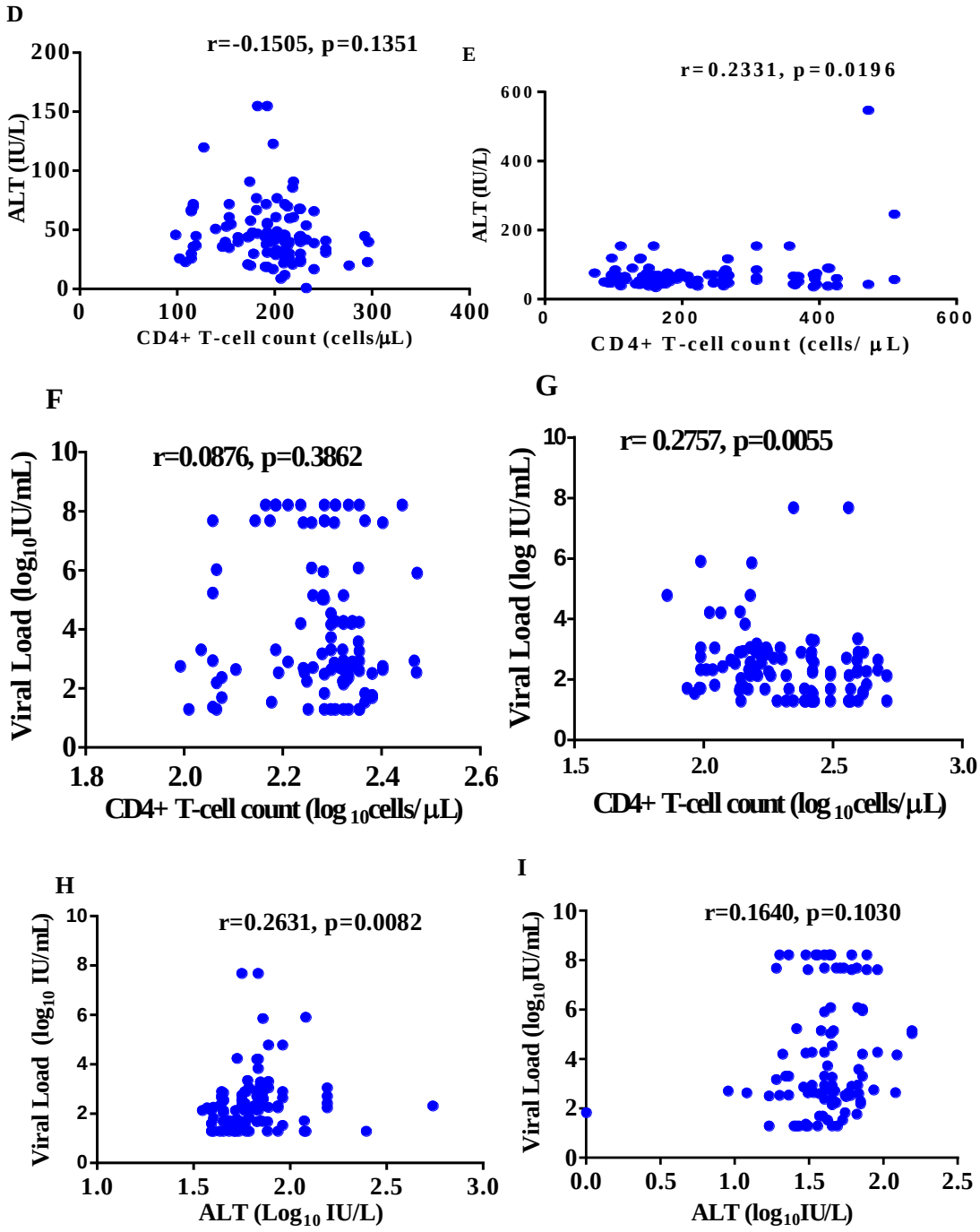


Figure 2. Correlation analysis between ALT, CD4 T-cell counts and HBV-DNA of the study participants. D represents the correlation between ALT and CD4+ T-cell count of HBV chronic infected study participants; E represents the correlation between CD4 T-cell count and ALT in acute HBV infected study participants; F represents the correlation between viral load (VL) and CD4+ T-cell count of HBV chronic infected participants; G represents the correlation between VL and CD4+ T-cell count of HBV acute infected participants; H represents the correlation between ALT and VL of HBV acute infected study participants and I represents the correlation between ALT and VL of chronic HBV infected participants (P≤ 0.05)



Discussion

Hepatitis B is a viral infection that attacks the liver and can cause both acute and chronic disease [24]. Despite widespread vaccination efforts, chronic HBV infection remains a significant global health challenge [25,26]. The present study explored how CD4+ T-cell counts, alanine aminotransferase (ALT) levels, and HBV-DNA interact in hepatitis B patients, distinguishing

between acute and chronic cases. Elevated ALT levels were linked to acute cases, while low CD4+ T-cell counts were prevalent in chronic cases. This highlights distinct clinical traits in different phases of HBV infection, potentially impacting patient care strategies [2].

The study compared ALT levels, CD4+ T-cell counts, and viral loads in acute and chronic hepatitis B virus (HBV) infections. ALT levels were highest in acute cases due to initial inflammation, while chronic cases showed lower, more stable levels [26,27]. Acute HBV participants had higher CD4+ T-cell counts, while chronic cases had lower counts, indicating a compromised immune response that might sustain the infection [27]. Chronic cases also exhibited significantly higher HBV-DNA levels, suggesting increased viral replication and activity, contributing to the persistence of chronic HBV infections [28].

The correlation between alanine aminotransferase (ALT), CD4+ T-cell counts, and HBV-DNA levels among individuals with hepatitis B virus (HBV) infection in our study provide valuable insights into their complex interplay. The study found a weak negative correlation between ALT and CD4+ T-cell counts in chronic HBV-infected individuals, which is consistent with some prior studies that hinted at a potential association between liver enzyme levels and immune response in chronic hepatitis B [7,8, 29]. The study found a moderate positive correlation between CD4+ T-cell counts and HBV-DNA levels in acute HBV-infected participants, with statistical significance, which adds statistical robustness to the association between immune response and viral replication during acute infection. This correlation aligns with and strengthens observations from previous studies [28]. As CD4+ T-cell counts increase, there is a corresponding elevation in HBV-DNA levels during the acute phase of infection. This finding could potentially inform future research on therapeutic interventions targeting CD4+ T-cell responses in acute HBV infection [30].

The study also found a differential impact of CD4+ T-cell counts on viral load dynamics in chronic and acute hepatitis B virus (HBV) infections, with a weak negative correlation in chronic HBV-infected individuals and a moderate positive correlation in acute infections. The weak negative correlation between CD4+ T-cell counts and viral load in chronic HBV-infected individuals aligns with some previous studies that have suggested a complex relationship between immune response and viral replication in chronic hepatitis B. A study by Boni *et al*. [31] proposed that while HBV-specific CD8+ T-cell responses play a crucial role in controlling chronic infection, the contribution of CD4+ T cells might be more nuanced and context-dependent. The weak negative correlation in chronic infection in our study may reflect the intricate balance between immune control and viral persistence. The moderate positive correlation between CD4+ T-cell counts and viral load in acute HBV-infected participants is an interesting contrast to the findings in chronic infection. This aligns with studies that have highlighted the dynamic nature of the immune response during the acute phase of HBV infection [32]. A study by Tan *et al*. [33] proposed that the early activation of CD4+ T cells is associated with enhanced viral replication during acute hepatitis B. Our findings lend support to the notion that the role of CD4+ T cells in modulating viral load may vary depending on the stage of infection [34].

The identification of a moderate positive correlation between alanine aminotransferase (ALT) and viral load in acute hepatitis B virus (HBV) infection, accompanied by statistical significance, is a noteworthy finding that resonates with the existing literature on the relationship between liver enzymes and viral replication during the acute phase of HBV infection. Several previous studies have investigated the association between ALT levels and viral load in different stages of HBV infection [18]. Elevated ALT levels are generally considered an indicator of liver inflammation and damage, often attributed to the host immune response against the virus [19]. The correlation observed in our study aligns with the notion that during the acute phase of HBV infection, there is active viral replication, leading to hepatocellular injury and subsequent release of ALT into the bloodstream [23]. One study by Ghany *et al*. [35] demonstrated a positive correlation between ALT levels and viral load during acute exacerbations of chronic hepatitis B. The findings suggested that ALT flares are associated with increased viral replication, reinforcing the idea that ALT can serve as a surrogate marker for ongoing viral activity [30].

Additionally, a meta-analysis conducted by Lok and McMahon [36] explored the relationship between ALT levels and viral load in chronic hepatitis B. While their focus was primarily on chronic infection, the study highlighted the general

association between elevated ALT and higher viral replication levels. Our study's alignment with this existing knowledge further supports the consistency of the observed correlation, emphasizing its relevance in acute HBV infection.

However, it is crucial to note that the dynamics of ALT and viral load correlation can vary across different phases of HBV infection. For example, during the chronic phase, the relationship may be more complex, with fluctuations in ALT levels not always mirroring changes in viral load. This complexity was acknowledged in a study by Han et al. [37], which emphasized the need for a nuanced interpretation of ALT levels in the context of chronic HBV infection.

Conclusion

This study shows a link between HBV-DNA, ALT levels, and CD4+ T-cell counts in hepatitis B virus infections. Low CD4+ T-cell counts indicate reduced immunity in chronic patients, but increased ALT levels indicate an inflammatory response in acute cases. CD4+ T-cell counts influence viral load differently in both acute and chronic stages, while ALT indicates viral activity during acute infection. A potential therapeutic target has been identified in acute infections, but due to the intricacy of ALT and viral load variations in chronic infections, more nuanced interpretation is required.

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Author contributions

Bukhari .I. Shuaib: Conceptualization, literature search, investigation methodology, formal analysis, writing, figures, writing—review and editing; Omosigho .O. Pius: literature search, methodology, formal analysis, writing, figures, writing—review and editing; Mathew F. Olaniyan: Investigation, methodology, writing—review and editing

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None

Conflicts of interest

The authors declare that they do not have any conflict of interest.

Data availability statement

Not Applicable

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