European Journal of Clinical Pharmacy
Print ISSN: 2385-409X **Online ISSN**: Applied Website:
https://farmclin.com/

CORRELATION OF HEPATITIS B VIRAL LOAD WITH LIVER FUNCTION TESTS IN PATIENTS WITH HEPATITIS B VIRUS

Shefali Singh, Dr.Supriya Mahajan*, Dr.Zarine Khan, ,Dr.Dalip K Kakru

PG Student1, Department of Microbiology, SMS & R, Sharda hospital,Greater Noida, India.
Associate Professor2, Department of Microbiology, SMS & R, Sharda hospital,Greater Noida, India.
Associate Professor3, Department of Microbiology, SMS & R, Sharda hospital,Greater Noida, India.
Professor and Head4,Department of Microbiology, SMS & R, Sharda hospital,Greater Noida, India.

Corresponding Author: Dr.Supriya Mahajan*
ShefaliSingh : shefalirajput9616@gmail.com
Dr.Supriya Mahajan: drsupriyamahajan@gmail.com
Dr.Zarine Khan: zarine.khan@sharda.ac.in
Dr.Dalip K Kakru: dalip.kakru@sharda.ac.in

ABSTRACT

Background and Objective:

Hepatitis Hepatitis B virus (HBV) infection remains a major global health concern, with chronic infection leading to significant morbidity and long-term liver complications. Liver Function Tests (LFTs) are routinely used to assess hepatic injury, while HBV DNA viral load reflects viral replication. However, the relationship between viral replication and biochemical liver injury remains unclear in clinical practice. This study aimed to evaluate the correlation between HBV viral load and liver function parameters, including in patients with HBV infection.

Methods:

A cross-sectional study was conducted over 12 months at a tertiary care center. A total of 142 HBsAg-reactive serum samples were included. HBV viral load was quantified using real-time PCR (TRUPC/F HBV Viral Load Kit). Corresponding LFT values were obtained from the Laboratory Information System. Statistical analysis was performed using SPSS version 23.0, and regression analysis was used to assess correlations between viral load and biochemical parameters.

Results:

The study population (n = 142) showed male predominance (65.4%). The median HBV viral load was 44 IU/mL, with 32.3% of patients having viral loads $<10^3$ IU/mL and 27.4% having viral loads $>10^6$ IU/mL. No statistically significant correlation was observed between HBV viral load and AST ($r = -0.139$, $p = 0.392$), ALT ($r = -0.157$, $p = 0.334$), ALP ($r = 0.040$, $p = 0.805$), TSB ($r = -0.139$, $p = 0.393$), direct or indirect bilirubin, or total protein. Notably, 23.9% of patients had high viral loads with normal LFTs, while 26.8% had low viral loads despite abnormal LFTs.

Interpretation and Conclusion:

The findings demonstrate a dissociation between HBV replication and biochemical liver injury. Viral load and hepatocyte damage represent independent aspects of HBV disease progression, emphasizing the need for combined virological and biochemical assessment for accurate clinical management.

KEYWORDS: Hepatitis B virus, Viral load, Liver function tests, Alanine transaminase, Aspartate transaminase , real time PCR.

How to Cite: Shefali Singh, Dr.Supriya Mahajan*, Dr.Zarine Khan, ,Dr.Dalip K Kakru, (2026) CORRELATION OF HEPATITIS B VIRAL LOAD WITH LIVER FUNCTION TESTS IN PATIENTS WITH HEPATITIS B VIRUS, European Journal of Clinical Pharmacy, Vol.8, No.1, pp. 138-146

INTRODUCTION

Hepatitis is an inflammatory liver disease characterized by the infiltration of inflammatory cells into hepatic tissue, a process that can lead to progressive fibrosis and potentially cirrhosis.[1] Hepatitis has both infectious and non-infectious etiologies, with viral forms—notably hepatitis A (HAV), B (HBV), C (HCV), D (HDV), and E (HEV)—being the most prevalent. Among these, HBV and HCV are particularly significant due to their tendency to establish chronic, often asymptomatic infections. Hepatitis B represents a substantial global health burden, with an estimated 300 million individuals affected and approximately one million deaths annually resulting from its complications.[2,3]

Hepatitis B virus (HBV), a small, enveloped DNA virus of the Hepadnaviridae family, was identified by Baruch Blumberg in 1965. The virion measures approximately 42 nm in diameter and consists of a 27 nm icosahedral nucleocapsid core surrounded by a host-derived lipoprotein envelope containing the hepatitis B envelope antigen (HBeAg).[2]

Globally, HBV endemicity is classified as high (>8%), intermediate (2–8%), or low (<2%) based on HBsAg prevalence. India, with an average prevalence of 3–4%, falls into the intermediate category. The country harbors 40 million HBV carriers, representing 10–15% of the global burden, of whom 15–20% progress to cirrhosis—contributing to significant morbidity, healthcare costs, and infant mortality.[4,5]

Hepatocellular function is assessed through biomarkers including serum aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and total bilirubin. ALT is the most specific marker for hepatic injury in viral hepatitis, owing to its predominantly cytoplasmic location in hepatocytes and longer serum half-life (~50 hours). In contrast, AST is less liver-specific due to its widespread tissue distribution. During early HBV infection, ALT typically exceeds AST; however, in chronic or advanced disease, AST levels may surpass ALT, reflecting mitochondrial damage. ALP is often normal or mildly elevated in acute viral hepatitis, while elevated serum bilirubin—particularly direct bilirubin—indicates hepatocellular dysfunction or cholestasis.[3]

MATERIALS AND METHODS

This 12-month cross-sectional study was conducted in the Central Laboratory of the Department of Microbiology, School of Medical Sciences and Research, Sharda Hospital, Sharda University, Greater Noida. The protocol was reviewed and approved by the institutional ethics committee. The study cohort comprised 142 consecutive, consenting patients with serologically confirmed Hepatitis B surface antigen (HBsAg) reactivity.

Rapid Immunochromatographic test (ICT) for HBsAg

Initial screening for Hepatitis B surface antigen (HBsAg) was performed using a commercial rapid immunochromatographic assay (HEPACARD, J. Mitra& Co. Pvt. Ltd., India), which employs a one-step sandwich immunoassay format. Following the manufacturer's protocol, 70 µl of serum was applied to the test device. Antigen present in the sample binds to colloidal gold-conjugated monoclonal antibodies, and the resulting complex is captured by immobilized polyclonal antibodies, producing a visible test line within 20 minutes. A valid internal control line confirmed proper test performance. Only samples with a valid positive result were included in the study.

HBV-DNA by real time PCR

Viral nucleic acid was extracted from serum samples using the silica-membrane-based TruPCR Viral Nucleic Acid Extraction Kit, following the manufacturer's protocol, which includes lysis, binding, washing, and elution steps. The purified DNA was subsequently quantified using the TRUPC/F HBV Viral Load real-time PCR kit. This assay employs specific primers and TaqMan probes targeting conserved regions of the HBV genome, along with an internal control and a pre-calibrated standard curve, enabling absolute quantification of HBV DNA viral load in International Units per milliliter (IU/mL). Amplification and analysis were performed in accordance with the manufacturer's instructions.

Data Collection

Liver function test results—including alanine aminotransferase (ALT), aspartate aminotransferase (AST), total serum bilirubin (TSB), direct bilirubin (DB), indirect bilirubin (InB), and alkaline phosphatase (ALP)—were retrieved from the institutional Laboratory Information System (LIS)

Statistical Analysis

Statistical analysis was done using the software SPSS version 23.0.The results were expressed as mean ± standard deviations (mean ± SD). Correlation between HBV viral load and individual parameters of liver function test results were carried out by scattered plot chart showing correlation coefficient.

RESULTS

Demographic characteristics of the study population: In Table 1, the study comprised 142 patients, with a male predominance [n=93, 65.4%] and a male-to-female ratio of 2:1. The median age was 46 years (range: 2–86). Most patients were inpatients (77%). Median liver function test values were within normal ranges: AST 48.0 U/L, ALT 44.0 U/L, ALP 108 U/L, total serum bilirubin 0.80 mg/dL, The median HBV viral load was 2.4×10^4 IU/mL, with a wide range from 29 IU/mL to 4.97×10^9 IU/mL with 4(5.33%) cases, followed by Surgical Intensive Care Unit (SICU) with 1 (1.33%) case.

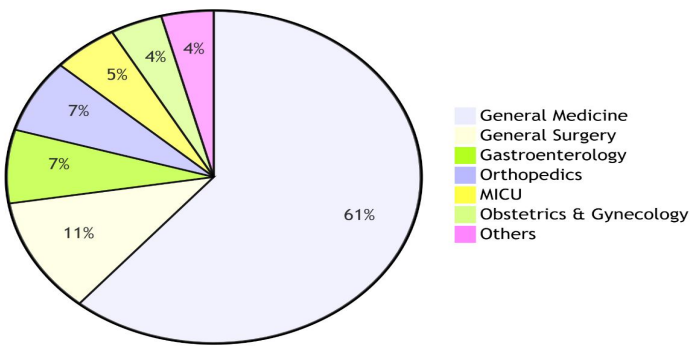
Table 1:Baseline characteristics of the study population

Total samples	142
Total males [n(%)]	93 (65.4%)

Total no. of females [n(%)]	49 (34%)	
Male:female ratio	2:1	
Median Age (years) (range)	46 (2- 86)	
Outpatients [n(%)]	48 (23%)	
Inpatients [n(%)]	94 (77%)	
Liver function markers	**Median**	**Normal rang**
Median Aspartate Transaminase (AST)	48.0 U/L	17 – 59 U/L
Median **Alanine Transaminase** (ALT)	44.0 U/L	00 – 50 U/L
Median **Alkaline Phosphatase (ALP)**	108 U/L	38 – 126 U/L
Total Serum Bilirubin (TSB)	0.80 mg /dl	0.20 – 1.3 mg/dl
Direct Bilirubin (DB)	0.31 mg/dl	0 – 0.4 mg/dl
Indirect Bilirubin (InB)	0.38 mg/dl	0 – 1.1 mg/dl
Total Protein (TP)	7.3 gm/dl	6.3 – 8.2 gm/dl
HBV viral load (median) (range)	2.4 x 10^4 IU/mL(2.9 x 10^1 IU/mL **to** 4.97 × 10^9 IU/mL)	

Distribution of patients: In Figure 1, **the** department-wise distribution of HBV-positive cases within the hospital setting. The majority of patients (61%) were managed by the Department of General Medicine. Other significant departments involved in patient care included General Surgery (7%), Gastroenterology (7%),smaller proportions were contributed by Orthopedics (6%), the Medical Intensive Care Unit (MICU) (5%), and Obstetrics &Gynecology (4%). The remaining 10% of cases were distributed across various other departments, indicating the wide clinical spectrum and multi-departmental involvement in the management of Hepatitis B infection.

Figure 1:Department-wise Distribution of HBV Patients (n = 142)



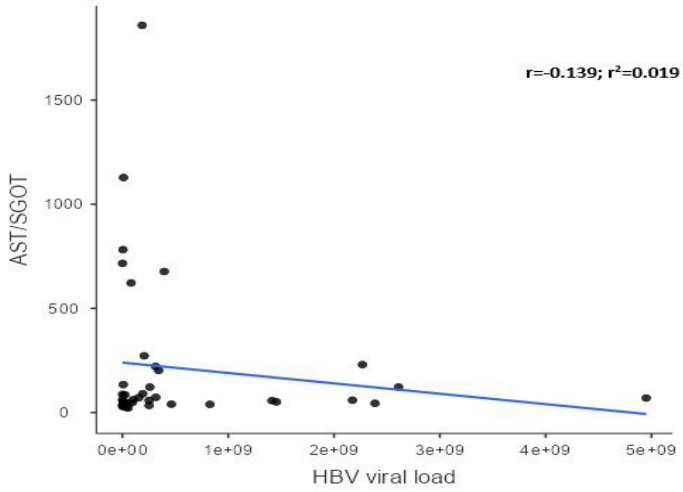
HBV DNA Viral Load Distribution in the Study Population (n = 142): In Table 2 The distribution of HBV viral load among the study varied widely. A substantial proportion of patients, 46 (32.3%), had low-level viremia (<10^3 IU/mL), while 41 (28.8%) fell within the 10^3–10^4 IU/mL range. Fewer patients, 16 (11.2%), exhibited moderate viral loads (10^4–10^6 IU/mL). Notably, a significant subset of 39 patients (27.4%) demonstrated high-level viremia exceeding 10^6 IU/mL.

Table 2: HBV DNA Viral Load Distribution in the Study Population (n = 142)

Viral Load Range (IU/mL)	Number of Patients [n (%)]
< 10^3	46 (32.3)
10^3 – 10^4	41 (28.8)
10^4 – 10^6	16 (11.2)
> 10^6	39 (27.4)
TOTAL	142 (100)

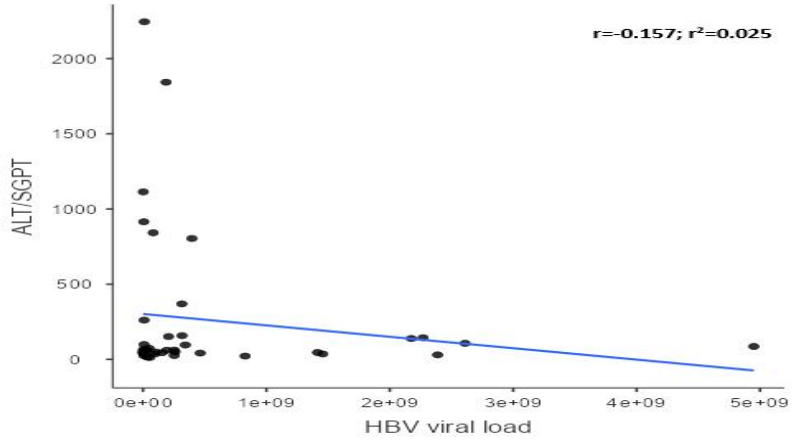
Correlation between HBV viral load and liver function test parameters; Correlation analysis revealed no statistically significant relationship between HBV viral load and any liver function parameter. Weak, non-significant correlations were observed with AST (r = -0.139, p = 0.392) (Figure 2), ALT (r = -0.157, p = 0.334) (Figure 3), ALP (r = 0.040, p = 0.805) (Figure 4) and total serum bilirubin (r = -0.139, p = 0.393) (Figure 5) Direct Bilirubin (DB) (r = -0.158, r^2 = 0.025, p = 0.330) [Figure. 6], Indirect Bilirubin (InB) (r = -0.034, r^2 = 0.001, p = 0.836) [Figure. 7], and Total Protein (r = -0.046, r^2 = 0.002, p = 0.779) [Figure. 8].The consistently low correlation coefficients and non-significant p-values indicate that HBV viral load does not reliably predict biochemical liver injury in this study.

Figure 2:Scattered plot chart showing correlation of HBVviral load with Aspartate Transaminase (AST) in study population (n =142)



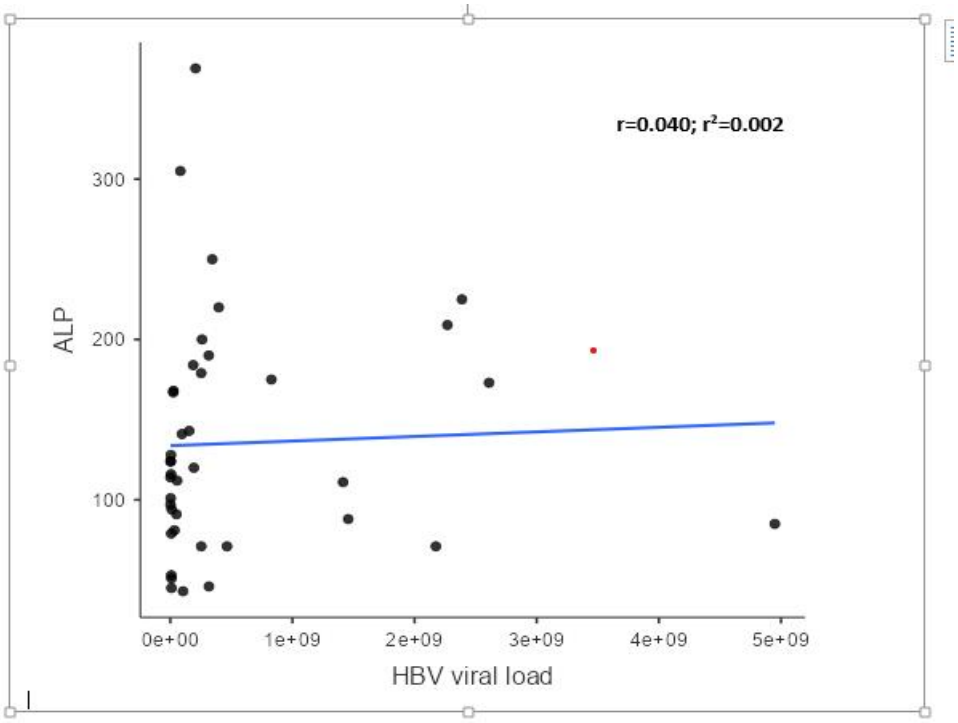
r = Correlation-0.139; r^2 = Correlation coefficient 0.019

Figure 3:Scattered plot chart showing correlation of HBVviral load withAlanine Transaminase (ALT)in study population (n =142)



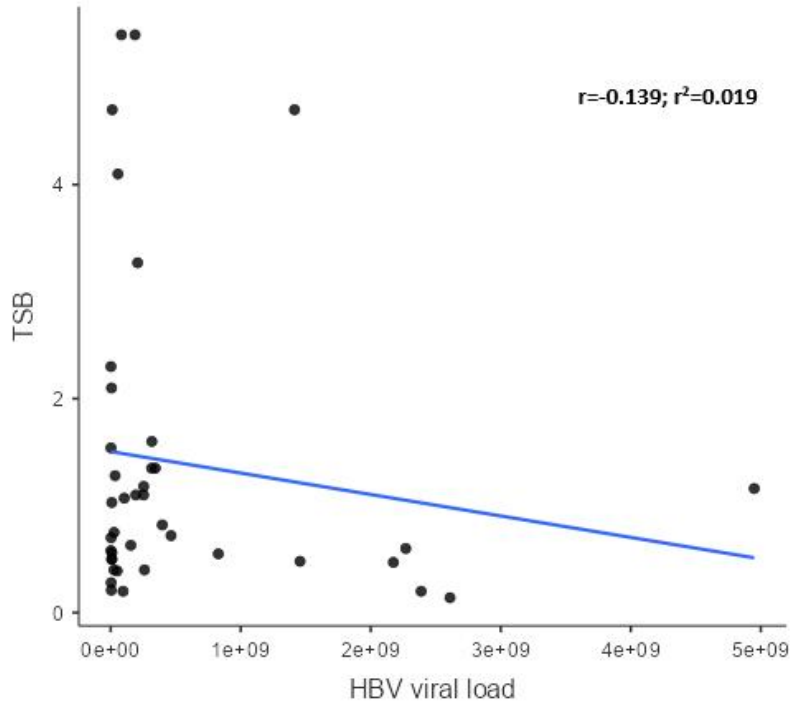
r = Correlation-0.157; r^2 = Correlation coefficient 0.025

Figure 4:Scattered plot chart showing correlation of HBV viral load Alkaline Phosphatase (ALP)in study population (n =142)



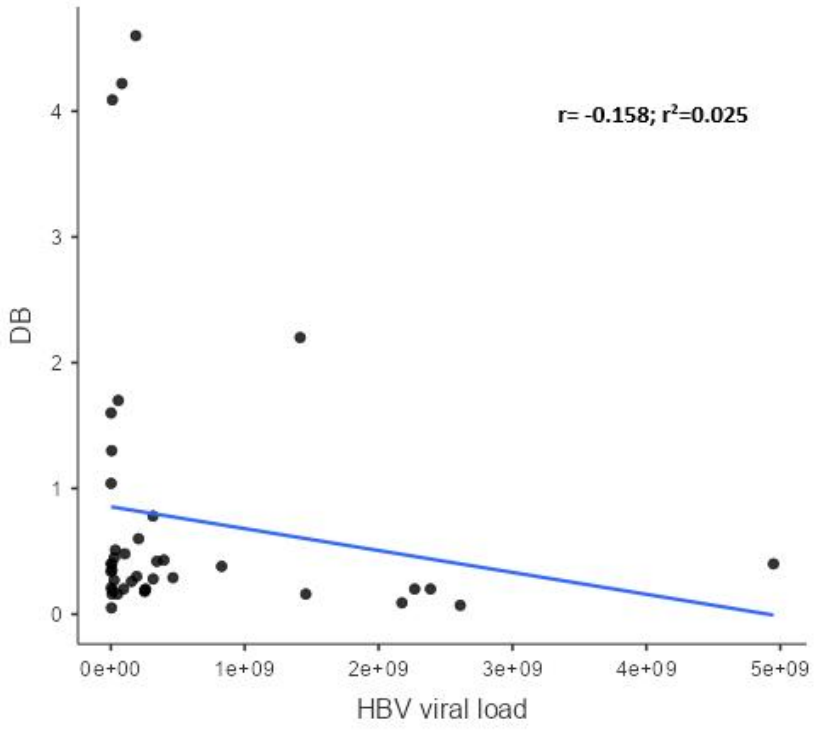
r = Correlation0.040; r^2 = Correlation coefficient 0.805

Figure 5 :Scattered plot chart showing correlation of HBV viral load with Total Serum Bilirubin (TSB)in study population (n =142)



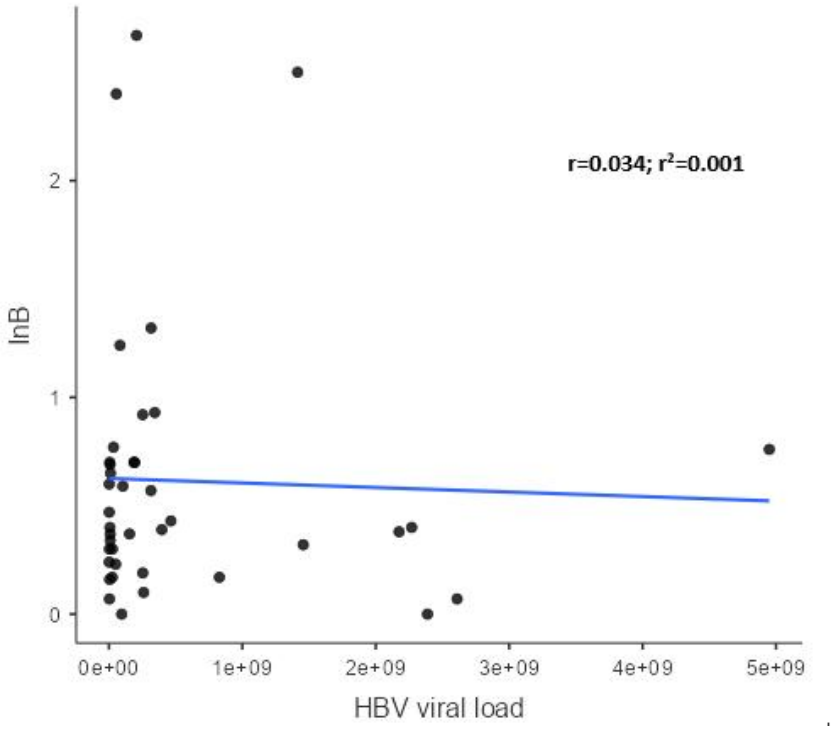
r = Correlation; -0.139 r^2 = Correlation coefficient 0.019

Figure 6:Scattered plot chart showing correlation of HBVviral load with Direct Bilirubin (DB)in study population (n =142)



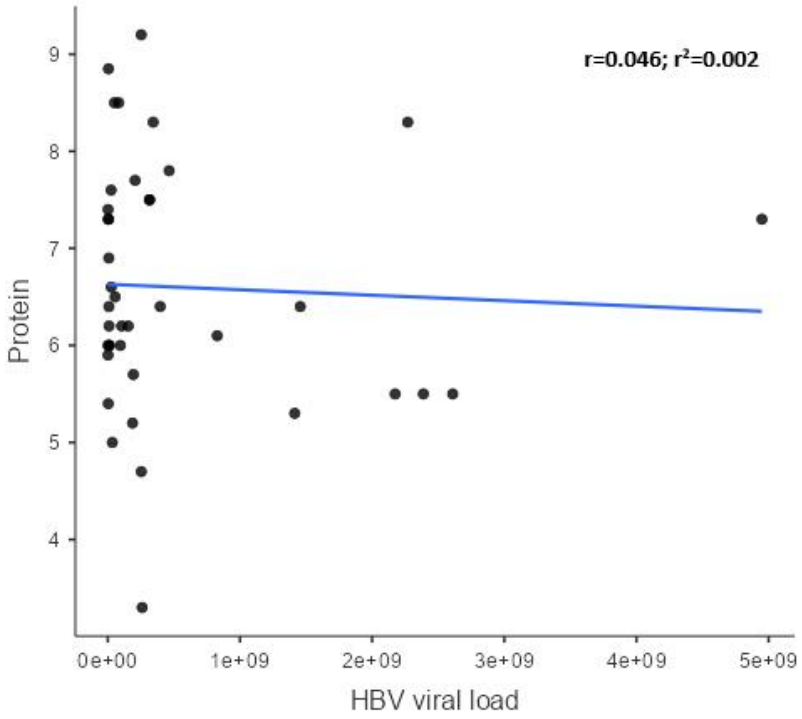
r = Correlation-0.158; r^2 = Correlation coefficient 0.025

Figure 7:Scattered plot chart showing correlation of HBVviral load Indirect Bilirubin (InB)in study population (n =142)



r = Correlation-0.034; r^2 = Correlation coefficient 0.836

Fig.8 correlation of HBV viral load with Total protein in HBV patient



r = Correlation -0.046, r^2 = Correlation coefficient 0.002

Overall conclusion of the study: This study of 142 Hepatitis B patients revealed a key finding that demonstrates a critical limitation in current clinical practice: relying exclusively on LFTs to assess hepatitis B severity is an inadequate approach which is unfortunately a mindset of many clinicians. Our analysis showed no consistent relationship between HBV viral load and LFT values, indicating that these common blood tests cannot reliably reflect the actual state of viral activity or liver damage. Patients may show normal LFT results despite active viral replication, or display abnormal LFT values with minimal viral presence. Therefore, using LFTs in isolation provides an incomplete and potentially misleading picture of the true activity of disease. A comprehensive assessment that integrates both virological and biochemical markers is essential for accurate clinical decision-making and effective patient management.

DISCUSSION

This study, comprising of a total 142 HBsAg reactive patients; was aimed to determine the correlation between HBV viral load and different LFTs seaparately, i.e. ALT, AST, ALP, TSB, DB, InB and TP. The study demonstrated male preponderence with a male-to-female ratio of 2:1, which is a characteristic frequently observed in epidemiological studies of HBV infection, potentially reflecting higher exposure risk among male population[6, 7, 8, 9, 10].

While LFTs are well-established markers of liver damage, our study found no significant correlation between their levels and HBV viral load. This result is in contrast with other studies which have shown positive correlation between HBV viral load and ALT/AST [1 , 11, 12]. Altom et al [12] reported strong positive correlations between HBV DNA load and transaminases, ALP, and bilirubin, alongside a negative correlation with albumin. The authors interpreted this pattern as direct evidence linking active viral replication to hepatocellular injury, biliary involvement, and impaired synthetic function within their population. These studies have given the reasoning that higher viral load leads to destruction of these hepatocytes which further leads to release of these enzymes from the ruptured hepatocytes that can lead to abnormality of these enzymes [1].

Our findings, which demonstrate no significant correlation between serum HBV DNA levels and liver function test parameters, align with the observations of Biazar et al. [13]. It can be presumed that the degree of viral replication does not reliably predict the extent of hepatocellular injury in hepatitis B infection. Together, these results emphasize the distinct and often dissociated roles of virological and biochemical biomarkers in chronic hepatitis B, reinforcing the necessity of integrating direct viral load assessment into clinical protocols, irrespective of liver enzyme profiles.

According to Fields Virology (6th Edition) [14], there are four stages of chronic hepatitis B i.e. **Immune-Tolerant phase**, the **Immune-Active (or Immune-Clearance) phase**, the **Inactive Carrier phase,** and the **Reactivation phase**. Immune tolerant stage is the first stage which is characterized by high serum HBV DNA levels (10^9 - 10^12),alongside normal liver enzymes (ALT/AST) and minimal liver inflammation. This state is actively maintained by the hepatitis B e antigen (HBeAg), which suppresses the host's immune response, creating a tolerant environment that permits extensive viral replication without

significant hepatocellular injury. This immunotolerant phase can persist for several decades. In the second stage which is immune clearance phase, HBV viral load is moderately high (>2000 - <10^9) but decreased as compared to stage 1 this is because during the immune-tolerant phase, the body's immune system largely ignores the hepatitis B virus, allowing it to replicate freely, resulting in a very high viral load. The transition to the immune-clearance phase marks a critical shift where the immune system finally recognizes the infection and launches an attack. Activated immune cells, particularly T-cells, start to both destroy infected liver cells and, more importantly, release powerful **Releasing cytokines** (like interferon) that suppress the virus's ability to replicate inside surviving cells. This direct immune pressure is what successfully reduces the viral load from its previous peak to more moderate levels. Whereas LFTs are now elevated showing a distinct pattern of active injury. **ALT is significantly elevated**, reflecting ongoing immune attack and death of infected liver cells. **Bilirubin may also rise**, signaling more severe damage. While enzymes like ALP and GGT can increase due to inflammation, the liver's synthetic function (albumin, clotting factors) usually remains normal unless damage becomes very severe. In short, the LFT profile acts as a real-time marker of the immune system's fight against the virus. Following HBeAg seroconversion, most patients enter the third stage which is the **inactive carrier stage**, characterized by normal ALT, normal bilirubin, and very low to undetectable HBV DNA (<2000); indicating biochemical remission and a generally benign course. However, **20-30% reach the fourth stage i.e. reactivation**, with renewed elevation in ALT and HBV DNA (>2000), which can lead to progressive liver injury. Even with normal LFTs, carriers—particularly those with cirrhosis—retain a **low but ongoing risk of hepatocellular carcinoma.** **The clearance of HBsAg** represents the most favourable outcome, dramatically improving prognosis, although it does not fully eliminate risk in those with pre-existing advanced fibrosis. The immune-reactive phase of Hepatitis B reveals an important clinical observation where patients can show substantial liver injury with elevated liver function tests, particularly ALT, even when their HBV DNA levels are lower as compared to immune-tolerant stage. This pattern indicates that the extent of liver damage is not governed by viral load alone. Instead, the host's immune response, specifically a vigorous Th1-mediated attack on infected liver cells, propels the continuing liver inflammation and fibrosis. This relationship highlights the necessity of tracking both viral markers and liver enzyme levels, as a low viral load does not eliminate the possibility of serious liver disease during this active stage of HBV infection [14].

The findings from Fields Virology, 6th Edition [14] provide a strong virological basis for the lack of correlation between HBV viral load and liver function tests observed in our study. The textbook describes how the immune tolerance stage features high viral replication without liver damage (normal LFTs), while the reactive phase demonstrates significant liver injury (elevated LFTs) even with lower viral loads. This clear dissociation between viral activity and hepatocellular injury in established HBV disease stages directly aligns with and supports our results.

Conclusion

This study of 142 Hepatitis B patients revealed a key finding that demonstrates a critical limitation in current clinical practice: relying exclusively on LFTs to assess hepatitis B severity is an inadequate approach which is unfortunately a mindset of many clinicians. Our analysis showed no consistent relationship between HBV viral load and LFT values, indicating that these common blood tests cannot reliably reflect the actual state of viral activity or liver damage. Patients may show normal LFT results despite active viral replication, or display abnormal LFT values with minimal viral presence. Therefore, using LFTs in isolation provides an incomplete and potentially misleading picture of the true activity of disease. A comprehensive assessment that integrates both virological and biochemical markers is essential for accurate clinical decision-making and effective patient management.

DECLARATIONS

Conflicts of interest: There is no any conflict of interest associated with this study.

Consent to participate: There is consent to participate**.**

Consent for publication: There is consent for the publication of this paper.

Authors' contributions: Author equally contributed the work.

REFRENCES

[1] Fazaa, Alaa Hameed, Ahmed K. Atya, and Husam M. Kredy. Evaluation of Liver Function Tests and Their Correlation with HBV Viral Load in Patients with Hepatitis B Virus, Thi-Qar, Iraq. HIV Nursing 2022; 22(2):1112-6.

[2] Karim SM, Rahman MR, Shermin S, Sultana R. Correlation between aminotransferase ratio (AST/ALT) and other biochemical parameters in chronic liver disease of viral origin. Delta Med Col J. 2015 Feb 14;3(1):1-5.

[3] Özdemir YE, Doğdaş ES, Demir AS, Borcak D, Ünlü EC, Sezen Aİ, et al. Relationships Between Liver Histopathology with Biochemical Parameters, and Hepatitis B Virus-DNA in Chronic Hepatitis B Infection. Viral Hepatitis Journal/Viral HepatitDergisi. 2023 Aug 1;29(2).

[4] https://wbhealth.gov.in/NVHCP/contents/page/background

[5] Premkumar M, Kumar Chawla Y. Chronic hepatitis B: challenges and successes in India. Clinical liver disease. 2021 Sep;18(3):111-6.

[6] Lim JK, Nguyen MH, Kim WR, Gish R, Perumalswami P, Jacobson IM. Prevalence of chronic hepatitis B virus

infection in the United States. Official journal of the American College of Gastroenterology| ACG. 2020 Sep 1;115(9):1429-38

[7] Soběslavský O. Prevalence of markers of hepatitis B virus infection in various countries: a WHO collaborative study. Bulletin of the World Health Organization. 1980;58(4):621

[8] Brown R, Goulder P, Matthews PC. Sexual dimorphism in chronic hepatitis B virus (HBV) infection: evidence to inform elimination efforts. Wellcome open research. 2022 May 11;7:32.

[9] Khan F, Shams S, Qureshi ID, Israr M, Khan H, Sarwar MT, Ilyas M. Hepatitis B virus infection among different sex and age groups in Pakistani Punjab. Virology journal. 2011 May 13;8(1):225

[10] Gupta D. Correlation Between HBV DNA Viral Load and Liver Function Test Parameters in Chronic Hepatitis B Patients: A Cross-Sectional Study from India.

[11] Takieddine FN, Tufenkeji HT, Sheth KV, Ghandour MH. Prevalence of HBV markers in adult Saudis in relation to age and sex. Annals of Saudi Medicine. 1986 Jul;6(3):205-9.

[12] Altom SA, Nail AM, Musa AM, Modawe GA. Correlation between Hepatitis B Viral Load and Liver Function Test among Hepatitis B Virus Patients in Khartoum State, Sudan. Sirte Journal of Medical Sciences. 2023 Dec 27;2(2):8-15.

[13] Biazar T, Yahyapour Y, Roushan MR, Rajabnia R, Sadeghi M, Taheri H, Ranaei M, Bayani M. Relationship between hepatitis B DNA viral load in the liver and its histology in patients with chronic hepatitis B. Caspian journal of internal medicine. 2015;6(4):209.

[14] Howley PM, Knipe DM,.Cohen JI, Griffin DE, Lamb RA, Martin MA, et al. Fields virology: 6th edition Lippincott Williams & Wilkin; 2013 May 21.