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Study of Viral Load Pattern among Hepatitis B Surface Antigen-Positive Patients in a Tertiary Care Hospital in Southern Rajasthan

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ABSTRACT

Background: Hepatitis B virus (HBV) infection remains a major global public health problem and is a leading cause of chronic liver disease, cirrhosis, and hepatocellular carcinoma. Quantitative estimation of HBV viral load is central to assessing disease activity, infectivity, prognosis, and eligibility for antiviral therapy. Regional data on viral load patterns among Hepatitis B surface antigen (HBsAg)–positive individuals are essential for optimizing clinical management and strengthening public health interventions.

Methods: A hospital-based cross-sectional study was conducted from January to July 2024 at RNT Medical College and Maharana Bhupal Government Hospital, Udaipur. Four hundred HBsAg-positive patients (≥18 years) were included. Demographic details were recorded, and HBV DNA was quantified using RT-PCR (Artus HBV RT PCR kit). Viral load (IU/mL) was categorized into standard clinical ranges and analyzed statistically.

Results: Of 400 patients, 65% were male and 35% female, predominantly aged 21–40 years. Undetectable HBV DNA was seen in 39.2%, low-level viremia (20–2,000 IU/mL) in 27.5%, and viral load >20,000 IU/mL in 17.8%. Viral load showed no significant association with gender (p=0.83) or age (p=0.77). Mean viral load was comparable across age groups (p=0.579). Most patients were from Udaipur district (46%; p<0.001).

Conclusion: Most HBsAg-positive patients exhibited low or undetectable viral loads, indicating a predominance of inactive or low-replicative infection. HBV DNA quantification remains essential for treatment stratification, and region-specific surveillance strategies are crucial for effective HBV control.

Key-words: Hepatitis B virus; HBV DNA; Viral load; Real-time PCR; Chronic hepatitis B; Epidemiology; Southern Rajasthan

INTRODUCTION

Hepatitis viruses constitute a heterogeneous group of viruses that are taxonomically diverse but share a common tropism for hepatocytes. Infection with these viruses results in acute liver inflammation, producing histopathological changes and overlapping clinical manifestations, including fever, nausea, vomiting, and jaundice [1].

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Viral hepatitis remains one of the most common causes of liver-related morbidity worldwide. Almost all cases of viral hepatitis are attributed to five major viral agents: hepatitis A virus (HAV), which causes infectious hepatitis; hepatitis B virus (HBV), responsible for serum hepatitis; hepatitis C virus (HCV), a common cause of post-transfusion hepatitis; hepatitis D virus (HDV), a defective virus that requires HBV for replication; and hepatitis E virus (HEV), the agent of enterically transmitted non-A, non-B hepatitis. Among these, all are RNA viruses except hepatitis B, which is a DNA virus that replicates via a reverse-transcription mechanism similar to that of retroviruses [1].

Hepatitis B virus infection represents a major global public health burden. It is estimated that over two billion people worldwide show serological evidence of past or

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present HBV infection, and approximately 350 million individuals are chronic carriers of the virus [2–5]. Chronic HBV infection is associated with significant long-term sequelae, including chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Certain regions demonstrate particularly high endemicity; for example, West Africa shows elevated prevalence rates, especially among infants, largely due to vertical transmission [2–5]. In Nigeria, reported infection rates range from 7.3% to 24%, reflecting substantial regional variation and underscoring the need for localized epidemiological data [3].

Hepatitis B virus is a hepatotropic virus capable of causing both acute and chronic liver disease with potentially fatal outcomes. It belongs to the family Hepadnaviridae, genus Orthohepadnavirus. This viral family also includes hepatitis viruses that infect lower animals, such as woodchucks, squirrels, and ducks. HBV was first identified in the serum of Australian aborigines in the 1960s. The antigen detected during these early studies was initially termed the “Australia antigen” by Blumberg and colleagues in 1965 and was frequently observed in patients with leukemia. This antigen is now recognized as hepatitis B surface antigen (HBsAg), a key serological marker of HBV infection. Subsequent research established hepatitis B as a widespread global disease, with more than two billion individuals exposed worldwide. According to the World Health Organization estimates, 296 million people were living with chronic HBV infection in 2019 [6].

Structurally, the hepatitis B virus is a DNA virus with a remarkably compact genome. Despite its small size of approximately 3.2 kilobases, HBV DNA encodes four overlapping open reading frames—S, C, P, and X—which produce multiple viral proteins. This overlapping gene organization allows HBV to maximize its coding capacity, representing an efficient genomic strategy. Once considered unique, HBV is now recognized as part of a broader family of hepatotropic DNA viruses, the hepadnaviruses [6-9].

HBV is an enveloped, partially double-stranded DNA virus with an unusual and complex life cycle. Following entry into hepatocytes through binding to the bile acid transporter sodium taurocholate cotransporting polypeptide (NTCP), the viral nucleocapsid releases relaxed circular DNA (rcDNA) into the cytoplasm. This rcDNA is transported to the nucleus, where host cellular enzymes convert it into covalently closed circular DNA (cccDNA). The cccDNA persists as a stable, non-integrated minichromosome and serves as the transcriptional template for all viral mRNAs. These transcripts are exported to the cytoplasm, where viral structural proteins and polymerase are synthesized. Viral replication occurs within nucleocapsids through reverse transcription of pregenomic RNA into new rcDNA. Mature nucleocapsids may either recycle rcDNA back to the nucleus to replenish the cccDNA pool or acquire an envelope and be secreted from the cell. In addition, infected hepatocytes release large quantities of noninfectious subviral particles composed primarily of HBsAg. Integration of HBV DNA into the host genome can result in continued HBsAg production even during antiviral therapy [7-12].

The clinical course of HBV infection is highly variable. Normal serum aminotransferase levels in individuals with detectable HBsAg may indicate an inactive carrier state, mild chronic hepatitis, or fluctuating disease activity. Measurement of hepatitis B e antigen and HBV DNA levels helps differentiate these phases, although these markers may also vary over time [6,13]. Assessment of viral load is therefore crucial for determining disease activity, prognosis, and treatment eligibility.

Transmission of hepatitis B occurs through multiple routes, including sexual contact, perinatal transmission, and percutaneous exposure. Sexual exposure remains a common mode of transmission, and a detailed sexual history is important when assessing risk. Vertical transmission can now be effectively prevented through passive and active immunization of newborns. Due to improved screening practices, transfusion-related transmission has become an uncommon cause of acute viral hepatitis. Overall, viral hepatitis continues to be a leading cause of acute and chronic liver disease [6,14].

In this context, studying HBV viral load patterns among HBsAg-positive patients provides valuable insight into disease burden, transmission dynamics, and treatment needs, particularly at the regional level.

MATERIALS AND METHODS

Study Design and Setting- This hospital-based cross-sectional observational study was conducted in the Department of Microbiology in collaboration with the Department of Medicine at Ravindra Nath Tagore Medical College and Maharana Bhupal Government

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Hospital, Udaipur, Southern Rajasthan, over six months from 13 January to 23 July 2024.

Study Population and Sample Size- Patients attending the tertiary care hospital who tested positive for hepatitis B surface antigen (HBsAg) and were referred for HBV DNA viral load testing were included. A total of 400 HBsAg-positive patients constituted the study population.

Inclusion Criteria

- Patients who were HBsAg positive
- Patients aged ≥18 years
- Patients who provided written informed consent

Exclusion Criteria

- Patients with co-infection with HIV or Hepatitis C virus
- Known cases of liver malignancy
- Patients unwilling to participate in the study

Data Collection- After obtaining informed consent, relevant demographic and clinical details were recorded using a structured pro forma. The data collected included:

- Age
- Gender
- Place of residence (district)
- Available liver function test parameters
- HBV DNA viral load results

Sample Collection and Processing- Venous blood samples were collected under aseptic precautions in plain vacutainers. Serum was separated by centrifugation and stored at 2–8°C until processing. If testing could not be performed within 24 hours, serum samples were stored at –70°C. All samples were transported in accordance with Category B (UN 3373) guidelines for infectious substances.

Detection of HbsAg- Initial screening for Hepatitis B surface antigen was performed using rapid diagnostic tests (RDTs) based on immunochromatographic principles, following manufacturer instructions.

HBV DNA Quantification- Quantitative estimation of HBV DNA was performed using real-time polymerase chain reaction (RT-PCR).

DNA Extraction - Viral DNA was extracted from serum samples using the Qiagen Viral DNA Extraction Kit (Qiagen, Germany) according to the manufacturer's protocol. The extracted DNA was eluted and stored at recommended conditions until amplification.

Real-Time PCR Assay- HBV DNA quantification was performed using the Artus HBV RG Real-Time PCR kit (Qiagen, Germany) on a CFX96 real-time PCR system (Bio-Rad, USA). The assay targeted a conserved region of the HBV genome. Internal controls and external quantification standards were included in each run to monitor PCR inhibition and ensure accuracy.

HBV DNA levels were expressed in International Units per milliliter (IU/mL). Samples with viral load below the assay detection limit were reported as Template Not Detected (TND).

Viral Load Categorization- Based on HBV DNA levels, patients were categorized into the following groups: Template Not Detected (TND); <20 IU/mL; 20–2,000 IU/mL; 2,001–20,000 IU/mL; 20,000 IU/mL. These categories were used for analysis and for assessing treatment eligibility.

Statistical Analysis- Data were entered into Microsoft Excel and analyzed using appropriate statistical software. Categorical variables were expressed as frequencies and percentages. Associations between viral load and demographic variables (age, gender, and district) were assessed using the Chi-square test. A p-value of <0.05 was considered statistically significant.

Ethical Considerations- The study was conducted after obtaining necessary permission from the institutional authorities. Written informed consent was obtained from all participants. Confidentiality of patient information was strictly maintained throughout the study.

RESULTS

A total of 400 HBsAg-positive patients were included in the study. The study population showed a clear male predominance (65%). Most patients were young and middle-aged adults (21–40 years), representing the economically productive population. Table 1 shows the demographic profile of the study participants.

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Table 1: Demographic Profile of the Study Participants (n=400)

Variable	Category	Number (%)
Gender	Male	260 (65)
	Female	140 (35)
Age Group (years)	≤20	32 (8)
	21–30	112 (28)
	31–40	98 (24.5)
	41–50	78 (19.5)
	51–60	40 (10)
	>60	40 (10)

Nearly two-thirds of patients (66.7%) had undetectable or low-level viremia, while 17.8% had viral loads exceeding 20,000 IU/mL, indicating eligibility for antiviral therapy based on current guidelines. Table 2 shows the overall distribution of HBV viral load levels among patients.

Table 2: Overall Distribution of HBV Viral Load Levels among Patients

Viral Load Category (IU/mL)	Number (%)
Template Not Detected (TND)	157 (39.2)
<20	8 (2.0)
20–2,000	110 (27.5)
2,001–20,000	54 (13.5)
>20,000	71 (17.8)
Total	400 (100)

Although males outnumbered females across all viral load categories, no statistically significant association was observed between gender and HBV viral load levels. Table 3 shows the association between viral load category and gender.

Table 3: Association between Viral Load Category and Gender

Viral Load Category	Female (n=140)	Male (n=260)	Odds Ratio (95% CI)
TND	53	104	0.51 (0.03–8.31)
<20	4	4	1.00 (0.05–22.18)
20–2,000	43	65	0.66 (0.04–10.86)
2,001–20,000	19	35	0.54 (0.03–9.18)
>20,000	23	47	0.49 (0.03–8.18)

χ^2=7.31, p=0.83

Most patients were aged ≥30 years, but viral load distribution did not differ significantly between age groups, indicating age-independent viral replication patterns. Table 4 shows viral load distribution across age groups.

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Table 4: Viral Load Distribution across Age Groups

Viral Load Category	<30 yrs (n=94)	≥30 yrs (n=306)	Total
TND	35	122	157
<20	3	5	8
20–2,000	29	81	110
2,001–20,000	12	42	54
>20,000	15	56	71
Total	94	306	400

χ^2=1.77, p=0.77

Although higher mean viral loads were observed in the 21–30 years and >60 years groups, the difference across age categories was not statistically significant. Table 5 shows the measurable HBV viral load (IU/mL) by age group.

Table 5: Measurable HBV Viral Load (IU/mL) by Age Group

Age Group (years)	Mean Viral Load	Range	Patients with Measurable VL
11–20	8.10×10^3	1,510 – 14,700	2
21–30	8.57×10^7	10 – 4.26×10^9	71
31–40	5.56×10^6	10 – 1.19×10^8	44
41–50	2.69×10^6	8 – 1.09×10^8	44
51–60	4.90×10^6	10 – 1.29×10^8	41
>60	1.99×10^7	1 – 6.68×10^8	41

ANOVA: F=0.76, p=0.57

A significant geographic clustering of cases was observed, with nearly half originating from the Udaipur district, highlighting regional concentration and referral patterns. Table 6 shows the district-wise distribution of HBV +ve patients.

Table 6: District-wise Distribution of HBV-Positive Patients

District	Number (%)	Cumulative (%)
Udaipur	184 (46)	46
Chittorgarh	68 (17)	63
Rajsamand	46 (11.5)	74.5
Pratapgarh	38 (9.5)	84
Dungarpur	15 (3.8)	87.8
Sirohi	15 (3.8)	91.6
Bhilwara	14 (3.5)	95.1
Banswara	14 (3.5)	98.6
Salumber	6 (1.5)	100

χ^2=565.65, p<0.001

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DISCUSSION

The present hospital-based cross-sectional study assessed the viral load pattern among HBsAg-positive patients attending a tertiary care hospital in Southern Rajasthan, with a clear male predominance (65%). Similar male preponderance has been reported in multiple Indian and international studies, which attribute this pattern to higher exposure of males to risk factors such as unsafe sexual practices, occupational exposure to blood, injectable drug use, and increased healthcare-seeking behavior among men [3,6]. The majority of patients were in the 21–40-year age group, which represents the most economically productive segment of the population. This age distribution suggests that horizontal transmission during adolescence or early adulthood plays a significant role in HBV transmission in this region [6]. Comparable age patterns have been documented in other hospital-based studies from India and developing countries [2–5].

The virological profile showed that 39.2% of patients had undetectable viral load, while 27.5% had low-level viremia (20–2,000 IU/mL). This indicates that a large proportion of HBsAg-positive individuals are either inactive carriers or have minimal viral replication. Only 17.8% of patients had HBV DNA levels exceeding 20,000 IU/mL, a key threshold for initiating antiviral therapy per national and WHO guidelines [5]. Similar distributions, with a predominance of low or undetectable viral load, have been reported in previous studies, reflecting the natural history of chronic HBV infection, in which many individuals remain in an immune-tolerant or inactive phase for prolonged periods [5,6].

Although males outnumbered females across all viral load categories, no statistically significant association between gender and viral load was observed (χ^2=7.31, p=0.83). This finding suggests that while males may be more frequently infected, viral replication intensity once infected is comparable between genders. Similar observations have been reported in studies from India and Africa, where gender differences were more pronounced in prevalence rather than in virological severity [3,5]. Biological factors, such as hormonal influences on the immune response, have been suggested, but the evidence remains inconclusive [6].

The comparison of viral load distribution between patients aged <30 years and ≥30 years revealed no statistically significant difference (χ^2=1.77, p=0.77). This indicates that HBV DNA levels are not strongly influenced by age alone. Although older patients are often considered at higher risk for disease progression, viral load at a single time point may not reflect cumulative liver injury. Similar findings have been documented in earlier studies, emphasizing that treatment decisions should be guided by viral load in conjunction with liver function and fibrosis assessment rather than age alone [5,6].

The highest mean viral loads were observed in the 21–30 years and >60 years age groups; however, this difference was not statistically significant (F=0.76, p=0.57). Higher viral loads in younger adults may reflect immune-tolerant or early immune-active phases of infection. At the same time, elevated values in elderly patients may indicate immune escape or reactivation of chronic infection [6]. The wide range of viral load values across all age groups highlights the heterogeneous nature of HBV infection and reinforces the importance of individualized patient assessment [5].

A statistically significant clustering of cases was observed, with 46% of patients originating from Udaipur district (χ^2=565.65, p<0.001). This may be attributed to higher population density, better diagnostic facilities, referral bias, and greater healthcare accessibility in Udaipur compared to neighboring districts. Similar geographic clustering has been reported in other regional studies, underscoring the importance of district-level surveillance and targeted public health interventions under the National Viral Hepatitis Control Program [3]. Focused screening, vaccination, and awareness programs in high-burden districts are essential to reduce transmission and disease burden.

Collectively, the findings of this study align with the known epidemiology and natural history of chronic hepatitis B [14]. The predominance of low or undetectable viral load emphasizes the need for regular monitoring rather than universal treatment. In contrast, the minority with high viral load represents the key target group for antiviral therapy. Integration of molecular diagnostics with demographic and geographic data can strengthen HBV control strategies at the regional and national levels [5,6].

CONCLUSIONS

This hospital-based cross-sectional study provides valuable insights into the virological and demographic

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profile of Hepatitis B virus infection among HBsAg-positive patients attending a tertiary care center in Southern Rajasthan. A clear male predominance and higher representation of young and middle-aged adults were observed, reflecting the influence of behavioral, occupational, and socio-cultural factors on HBV transmission. Virological assessment revealed that the majority of patients had undetectable or low-level viremia, indicating an inactive or minimally replicative infection, whereas fewer than one-fifth met the recommended viral load threshold for initiating antiviral therapy. Importantly, no statistically significant association was found between viral load levels and age or gender, highlighting that viral replication is independent of these demographic variables. The significant geographic clustering of cases, particularly from the Udaipur district, underscores the role of referral patterns and regional healthcare accessibility. Overall, the findings emphasize the importance of HBV DNA quantification in guiding individualized patient management and support targeted surveillance, screening, and prevention strategies under the National Viral Hepatitis Control Program.

CONTRIBUTION OF AUTHORS

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