



COMPREHENSIVE PROFILING OF HEPATITIS B PATIENTS IN SOUTHERN RAJASTHAN: SEROLOGICAL, MOLECULAR, AND BIOCHEMICAL PERSPECTIVES

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ABSTRACT

Background: Hepatitis B virus (HBV) infection continues to be a major health problem across the world, especially in developing countries. Routine assessment of hepatitis B surface antigen (HBsAg) positive persons is needed to guide HBV management and treatment. This study was undertaken to find out the seroprevalence of HBsAg and correlation of serological, molecular and biochemical markers in HBsAg positive patients in Udaipur, Rajasthan. **Material and Methods:** This study was conducted on 16,204 suspected patients, whose blood samples were received in microbiology laboratory for HBsAg screening by rapid card test from various OPDs and IPDs of Maharana Bhupal government hospital and allied hospitals, Udaipur. All the HBsAg positive patients were further followed for the detection of hepatitis B envelope antigen (HBeAg) by ELISA, HBV viral load by Abbott RealTime HBV assay and biochemical markers (ALT, AST). **Results:** The mean age of patients was 42 ± 16.5 years (range 8-84 years) and majority were males. HBsAg positivity was found 1% (167/16204). Out of 167 HBsAg positive patients, 114 (68.3 %) were males and 53 (31.7%) were females. Among HBsAg positive cases 18 (10.8%) were HBeAg positive and 149 (89.2%) were HBeAg negative. HBV DNA was detected in 145 (86.8%) cases with mean viral load $2.8 \times 107 \pm 1.3 \times 107$ IU/mL. Mean ALT and AST levels were 41.2 ± 45.1 IU/mL and 35.3 ± 34.2 IU/mL, respectively. HBV viral load and ALT levels were significantly higher in HBeAg positive patients than HBeAg negative patients. **Conclusion:** Significant association was found between HBeAg, HBV viral load and ALT. So, estimation of these in hepatitis B patients is essential to correlate with disease progression and are used for decisions to treat and subsequent monitoring.

KEYWORDS : HBV, HBsAg, HBeAg, HBV DNA

INTRODUCTION

Viral hepatitis has been a major public health problem for the past three decades despite the use of antiviral therapy, and availability of an effective vaccine. In 2022, an estimated 1.3 million people died from viral hepatitis, with hepatitis B accounting for 1.1 million of those deaths.¹

Hepatitis B virus (HBV) is a partially double-stranded enveloped DNA virus and belongs to the family *Hepadnaviridae*. The disease can manifest both in acute and chronic forms and varies from asymptomatic to symptomatic progressive disease, such as cirrhosis and hepatocellular carcinoma. The majority of the hepatitis B endemicity in low-medium resource countries (LMIC) are caused by the transmission of the disease from mother to child. Other additional factors for HBV transmission are intravenous (IV) drug abuse, occupational exposure to infected blood and blood products, having multiple sexual partners, and lack of awareness.²

Routine assessment of hepatitis B surface antigen (HBsAg) positive individual is required to guide HBV infection management and indicate the need for initiation of treatment. This generally includes assessment of various serological markers (HBeAg, anti-HBs and anti-HBc), measurement of HBV DNA levels and assessing severity of liver disease by liver enzymes such as alanine aminotransferase, aspartate aminotransferase (ALT, AST), non-invasive tests (NITs) such as aspartate aminotransferase (AST)-to-platelet ratio index (APRI), FIB-4, FibroScan and liver biopsy. HBV viral load correlate with disease progression and are used for decisions to treat and subsequent monitoring.³

The aim of the present study was to find out the prevalence of hepatitis B and observe correlation between serological, biochemical and molecular markers in hepatitis B patients in southern Rajasthan.

MATERIAL AND METHODS

Study Subjects

This cross-sectional study was conducted in the department of

Microbiology, RNT Medical college & associated Hospital, a tertiary care hospital in Udaipur, Rajasthan, after the approval for the study from the Institutional Review Board and Ethics Committee. The study participants were patients who registered at the OPDs or were admitted to the IPDs of the hospital and advised to undergo HBsAg screening irrespective of age and sex for a period of two months, June 2025 to July 2025.

After HBsAg screening patients who were seropositive for the presence of HBsAg were further tested for the detection of HBeAg and HBV viral load. All the data regarding the details of the patients (age, gender, outdoor patient no.) and past/present medical history, various pathological and biochemical findings were noted in the sample collection proforma (Requisition form).

Blood samples were collected from all the patients who came with lab requisitions for the testing, in plain vial for serology (for serum) and EDTA (ethylene diamine tetra acetic acid) vial for plasma for PCR. Samples were stored in freezer at -20 C till they were tested.

The criteria for patient selection were no history of ongoing treatment. Exclusion criteria were incomplete demographic details, patients with alcoholic liver disease and co-infections (HIV, HAV, HCV and HEV), autoimmune liver diseases.

Serological and Biochemical Parameters

Screening for HBsAg was performed using a one step HBsAg rapid card test (Sidak Life Care Pvt Ltd). All the HBsAg positive samples were further tested for qualitative detection of HBeAg by ELISA kit (Real gene). All the tests were performed in accordance with the manufacturer's instructions with adequate controls.

Data regarding levels of AST, ALT, were taken from sample requisition forms for the study, which were considered elevated as per the manufacturer's protocol.

Extraction and Quantification of HBV DNA

Extraction, amplification and subsequent quantification of

HBV viral DNA was done by Abbott RealTime HBV Assay with the Abbott m2000sp and m2000rt instruments (Abbott Molecular Inc., IL, USA) as per the manufacturer's instructions. Assay results were reported in IU/mL or log IU/mL.

RESULTS

A total of 16,704 blood samples were received in the laboratory for the detection of HBsAg, of which 167 (1%) patients were seropositive for HBsAg. Among these HBsAg seropositive patients, 114 (68.3%) were males and 53 (31.7%) were females with mean age of 42 ± 16.5 years (range 8-84 years). In HBsAg positive patients the seropositivity of HBeAg was found to be 10.8% (18), while 89.2% (149) were 'HBeAg negative'. Out of these HBeAg positive patients 12 (66.7%) were males and 6 (33.3%) were females with HBeAg positivity rate of 10.5% and 11.3% in males and females, respectively. HBV DNA was detected in 145 (86.8%) cases with mean viral load $2.8 \times 10^7 \pm 1.3 \times 10^7$ IU/mL. In 22 patients target was not detectable and all were HBeAg negative. The mean of ALT and AST among patients were 41.2 ± 45.1 and 35.3 ± 34.2 IU/L respectively. (table 1)

It was seen that 14 (77.8%) out of 18 HBeAg positive patients were having viral load > 20000 IU/mL and 4 (22.2%) were having viral load < 20000 IU/mL. Out of 127 HBeAg negative samples 24 (18.9%) were having viral load > 20000 IU/mL and 103 (81.1%) were having viral load < 20000 IU/mL. (table 2) The mean viral load in HBeAg positive patients was $2.0 \times 10^8 \pm 3.2 \times 10^8$ IU/mL and in HBeAg negative patients $5.4 \times 10^6 \pm 4.8 \times 10^7$ IU/mL. A positive correlation was observed between HBeAg positivity and serum HBV DNA levels ($p < 0.05$). ALT levels were also higher in HBeAg positive patients (102.4 ± 150.2) as compared to HBeAg negative patients (53.6 ± 19.8).

Table 1 Demographic Profile, Hepatitis be Antigen Status, Virological and Biochemical Information of HBsAg Positive Patients

Variables	Population (n= 167)
Age, mean years $\pm$ SD	42 ± 16.5
Male/Female, (%)	114/53 (68.3/31.7)
HBeAg	
Positive (%)	18 (10.8)
Negative (%)	149 (89.2)
HBV DNA positive (%)	145 (86.8)
HBV viral load (IU/mL)	$2.8 \times 10^7 \pm 1.3 \times 10^7$
Range (IU/mL)	$8-1 \times 10^9$
ALT (IU/L)	41.2 ± 45.1
AST (IU/L)	35.3 ± 34.2

Data are presented as N (%) or Mean $\pm$ Standard Deviation

Table 2 HBeAg Status According to HBV Viral Load

Viral load (IU/mL)	Total (%)	HBeAg positive (%)	HBeAg negative (%)
TND*	22 (13.2)	-	22 (100)
< 2000	84 (50.3)	1 (1.2)	83 (98.8)
2000-20000	23 (13.8)	3 (13.0)	20 (87.0)
20000- 106	16 (9.6)	1 (6.3)	15 (93.7)
106-107	6 (3.5)	3 (50)	3 (50)
> 107	16 (9.6)	10 (62.5)	6 (37.5)
	167 (100)	18 (10.8)	149 (89.2)

*Target not detected

DISCUSSION

India is a country with intermediate to high endemicity of Hepatitis B surface antigen (HBsAg). Epidemiological updates are crucial to monitor the progress towards the global commitment to eliminate hepatitis by 2030.

In our study the seroprevalence of HBsAg was found to be 1%. This seroprevalence is in concordance with some earlier reports by Mathur et al. (2016) and Shadaker et al. (2022),

where the seroprevalence was 0.94% and 1.4% respectively.^{4,5}

In present study mean age was found to be 42 ± 16.5 years and majority of patients were adults (97%; 40.7% young adults, 41.9% middle age adults). To effectively target this adult population, it is imperative to implement comprehensive HBV awareness initiatives across educational institutions, workplaces, and other relevant settings. Furthermore, the initiation of adolescent vaccination programs is critical for the long-term reduction of new HBV infections.⁶ Gender analysis revealed that the prevalence of HBV was significantly higher in males than in females (68.3%/31.7%). Similar findings have been reported from various studies (Mathur et al. 2016, Attaullah et al. 2011, Nair et al. 2019).^{4,7,8} This could be due to males have more exposure to the risk factors than females because of occupation and other outdoor activities.

Serum HBeAg and HBV DNA both are the markers of active viral replication and high infectivity in hepatitis B patients. The levels of HBV DNA are associated with disease progression and assist in distinguishing active HBeAg-negative disease from inactive chronic infection, as well as guiding treatment decisions and monitoring responses to antiviral therapy; a rise may indicate the emergence of resistant variants.³ In the current study, 10.8% of patients tested positive for HBeAg, and HBV DNA was present in 86.8% of patients. The mean viral load was $2.8 \times 10^7 \pm 1.3 \times 10^7$ IU/mL in our study which aligns with the findings from studies done by Primadharsini et al (2013), Nirmala et al. (2023).^{9,10}

In 22 patients HBV DNA was not detected but they were HBsAg positive by rapid card test. This could be due to false-positive immunoassay reactions as real-time PCR is more sensitive assay in comparison to rapid card test, transient antigenemia post-vaccination, or persistent surface antigen expression without replication due to HBV genome integration.¹¹

The levels of HBV DNA were compared in HBeAg positive and HBeAg negative in our study (table 2). The results showed that all the HBeAg positive cases were also positive for HBV DNA and the viral load ranged from 563 IU / ml to > 10000000000 IU/mL and most of them had high viremic levels $> 20,000$ IU/mL. This is in accordance with the studies by Nair et al. (2019), Borgaonkar et al. (2019), Pandey et al. (2025).^{8,12,13}

In present study we observed that the HBV DNA load was high in HBeAg positive cases, and good correlation was seen between the two, but in 16.5% of HBeAg seronegative cases the HBV DNA levels were $> 20,000$ IU/mL, which could be due to mutations in the pre-core promoter region that abolish HBeAg production.^{13,14} According to NVHCP guidelines HBV viral load 20,000 IU/mL or more is the level at which treatment is started, and monitoring of these patients only on basis of HBeAg can be misleading in such cases.³ Thus, HBeAg is useful in diagnosis and treatment of Hepatitis B infection but it cannot replace HBV DNA specially when we consider HBeAg negative chronic hepatitis B (CHB) patients. HBV DNA quantitation by real time PCR is a reliable, accurate & reproducible test which can be used to diagnose, understand the natural history and progression or regression of the disease and also actively guide and monitor the therapy.¹²

In our study ALT levels were higher in HBeAg positive patients (102.4 ± 150.2) as compared to HBeAg negative patients (53.6 ± 19.8). Similar findings were observed by Pandey et al. 2025 at Faridabad, Haryana and by Vyas et al. (2022) at Jaipur, Rajasthan. HBeAg positivity and higher levels of ALT and HBV DNA have been reported to be associated with a higher cumulative incidence of HCC.^{13,15}

There have been many limitations in our study. First, the study did not include clinical data and longitudinal follow up of patients, quantitation of HBsAg, detection of anti-HBs, anti-

HBe, IgM and IgG anti-HBc, which would indicate acute or chronic infection, past infection, recovery and phase of chronic hepatitis B infection. Another limitation of the study was that sequencing was not done to know the presence of pre-core and core promoter mutations that inhibit or decrease the production of HBeAg, which would have also help us understand the correlation better.

CONCLUSION

In conclusion, this study reveals a low hepatitis B virus (HBV) seroprevalence of 1% in this part of Rajasthan, highlighting key factors such as seroprevalence, affected age groups, gender distribution, and the relationship between serological, molecular, and biochemical markers. These findings provide valuable insights for an effective implementation of the national viral hepatitis control program (NVHCP). Regular HBV viral load testing is recommended for all HBsAg-positive individuals, regardless of HBeAg status, to improve patient monitoring and treatment. Strengthening immunization coverage, expanding access to care, and implementing targeted population screening and treatment strategies through decentralized efforts are essential steps toward effective HBV control and elimination.

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Conflicts of Interest: None

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