Research Article

# Seroprevalence of Hepatitis B Surface Antigen and Viral Load Profile Among Patients Attending A Tertiary Care Hospital in Delhi NCR Region: A Cross-Sectional Study

Gunjan Pandey[1] , Ashutosh Rawat[2] , Ritu Agarwal[3]

[1]Ph.D. Scholar, Department of Microbiology, Santosh Medical College & Hospital, Santosh Deemed to be University, Ghaziabad, NCR, Delhi
[2]Professor & Head, Department of Microbiology, Santosh Medical College & Hospital, Santosh Deemed to be University, Ghaziabad, NCR, Delhi
[3]Professor & Head, Department of Microbiology, GS Medical College & Hospital, Pilkhuwa, Hapur, UP
Corresponding Author:
Gunjan Pandey (Ph.D. Scholar)
Department of Microbiology, Santosh Medical College & Hospital, Ghaziabad, NCR Delhi. Email ID: Pandeygunjan16@gmail.com

**ABSTRACT**

**Background:** Hepatitis B virus (HBV) infection remains a significant public health challenge in India, with an estimated 3–4% of the population chronically infected. The Delhi National Capital Region (NCR), with its diverse population drawn from multiple states, represents an epidemiologically important catchment area for understanding HBV burden. However, comprehensive data integrating seroprevalence, serological markers of viral replication, and quantitative viral load profiling from this region remain limited.

**Objectives:** To determine the seroprevalence of HBsAg among patients attending a tertiary care hospital in the Delhi NCR region and to characterize the HBV DNA viral load profile in association with serological markers (HBeAg, anti-HBc IgM, anti-HBc IgG).

**Materials and Methods:** We conducted a hospital-based cross-sectional study from July 2024 to August 2025. We screened 1,500 blood samples for HBsAg using the Hepa Card rapid immunochromatographic test (J. Mitra & Co., New Delhi). HBsAg-positive samples were further evaluated for HBeAg and anti-HBc (IgM and IgG) by ELISA (Dia.Pro. Diagnostic, Italy). HBV DNA extraction was performed using QIAamp DNA Mini Kit (Qiagen, Germany), and viral load quantification was carried out using the Artus HBV RG PCR Kit on the Qiagen Rotor-Gene Q real-time PCR platform. Statistical analysis was performed using SPSS version 26.0.

**Results:** HBsAg seroprevalence was 3.4% (51/1,500), confirming intermediate endemicity. Males comprised 58.8% (30/51), with peak prevalence in the 42–51 years age group (21.6%). HBeAg positivity was 94.1% (48/51), indicating widespread active viral replication. Anti-HBc IgM positivity was 86.3% (44/51). Among 49 successfully tested samples, 63.3% (31/49) demonstrated high viral load (>20,000 IU/mL). Acute infections constituted 87.7% (43/49) and chronic infections 12.3% (6/49).

**Conclusion:** Delhi NCR demonstrates intermediate HBV endemicity with remarkably high rates of active viral replication. The majority of HBsAg-positive patients harbour substantial viral loads with concurrent hepatocellular injury, underscoring the need for early screening, viral load monitoring, and timely clinical intervention.

**Keywords:** Hepatitis B virus; HBsAg; Seroprevalence; Viral load; HBeAg; Delhi NCR; Real-time PCR.

## INTRODUCTION:

Hepatitis B virus (HBV) infection constitutes one of the most significant global public health challenges, affecting approximately 296 million individuals worldwide and accounting for nearly 820,000 deaths annually due to its complications, which include cirrhosis, hepatic decompensation, and hepatocellular carcinoma (HCC). The World Health Organization (WHO) has classified HBV infection as a major contributor to the global burden of chronic liver disease and has set ambitious targets for its elimination as a public health threat by the year 2030.[1]

India occupies a unique position in the global epidemiology of HBV infection. With an estimated 3–4% of the general population chronically infected, India falls within the intermediate endemicity zone, although significant regional variations exist. Population-weighted analyses have estimated an overall hepatitis B prevalence of approximately 3.7% in India, corresponding to a chronic carrier rate of about 2.96%.[2] The country is also estimated to harbour approximately 40 million chronic HBV carriers. The economic and clinical burden imposed by HBV-related morbidity and mortality in India is substantial, particularly in resource-limited settings where access to antiviral therapy and regular monitoring remains inconsistent.[3]

HBV is a partially double-stranded DNA virus belonging to the family Hepadnaviridae. The virus is transmitted through percutaneous or mucosal exposure to infected blood and body fluids, with the principal modes of transmission including perinatal (vertical) transmission, horizontal transmission during early childhood through close household contact, unsafe injection practices, sexual contact, and occupational exposure among healthcare workers.[3,4] In India, both horizontal and perinatal transmission play significant roles, with the relative contribution varying across different socio-economic and geographical strata.[3]

The serological diagnosis of HBV infection relies on a panel of markers, each of which provides specific information regarding the stage of infection, viral replication status, and infectivity. Hepatitis B surface antigen (HBsAg), the hallmark marker of HBV infection, is the first serological indicator to appear following exposure, and its persistence beyond six months defines the transition from acute to chronic infection. Hepatitis B e antigen (HBeAg) is a soluble secretory protein derived from proteolytic processing of the pre-core protein within hepatocytes and serves as a well-established surrogate marker of active viral replication and high infectivity.[4,5] Antibodies to the hepatitis B core antigen (anti-HBc) of the IgM class appear early in acute infection and serve to distinguish acute from chronic hepatitis B, while anti-HBc IgG typically persists for the lifetime of the individual and indicates prior or ongoing exposure.[4]

The quantification of HBV DNA viral load by real-time polymerase chain reaction (RT-PCR) represents a key method for assessing the level of viral replication and is integral to treatment decisions, monitoring of therapeutic response, and assessment of transmission risk.[6] High HBV DNA levels are associated with increased risk of disease progression, cirrhosis, and HCC, although treatment decisions should be based on HBV DNA together with ALT, disease stage, and other clinical factors.[6,7]

The Delhi National Capital Region (NCR) is one of the most densely populated and demographically diverse metropolitan areas in India, drawing its population from multiple states including Uttar Pradesh, Haryana, Rajasthan, and Delhi. Despite its epidemiological importance, comprehensive data integrating HBsAg seroprevalence with quantitative viral load profiling, serological characterization, and hepatic function assessment from this region remain conspicuously limited. Most available studies from India have focused on either seroprevalence or molecular characterization in isolation, without integrating these complementary dimensions of HBV disease assessment.

The present study was therefore designed to address this gap by determining the seroprevalence of HBsAg among patients attending a tertiary care hospital in the Delhi NCR region and characterizing the HBV DNA viral load profile in association with serological markers and liver function parameters, thereby providing a comprehensive epidemiological and virological snapshot of HBV infection in this population.

## MATERIALS AND METHODS:

**Study Design and Setting:** A hospital-based cross-sectional observational study was conducted in the Department of Microbiology at Santosh Medical College and Hospital, Santosh Deemed to be University, Ghaziabad, and in collaboration with the Department of Microbiology, GS Medical College and Hospital, Pilkhuwa, Hapur, Uttar Pradesh. A total of 1,500 patients were enrolled in the outpatient (OPD) and inpatient (IPD) departments during the study period. Patients of all age groups and both sexes were included. Patients on immunosuppressive drugs and those with a history of alcohol intake were excluded.

**Study Duration and Ethical Approval:** The study was conducted over a period of approximately two years, from July 2024 to August 2025. Ethical clearance was obtained from the Institutional Ethics Committee before commencement.

**Sample Collection and Processing:** Five millilitres of venous blood were collected from each participant under aseptic precautions into plain vacutainer tubes. Serum was separated by centrifugation at 4000 RPM for 10 minutes within 6 hours of collection and aliquoted into sterile microcentrifuge tubes. One aliquot was used for immediate testing; the second was stored at −80°C.

## Serological Investigations

**HBsAg Screening:** All 1,500 samples were screened for HBsAg using the Hepa Card rapid immunochromatographic test (J. Mitra and Co. Pvt. Ltd., New Delhi), a lateral flow sandwich immunoassay with a sensitivity of 0.5 ng/mL.

**HBeAg Detection:** HBsAg-positive samples were tested for HBeAg using an ELISA kit (Dia.Pro. Diagnostic Bioprobes S.r.l., Milan, Italy). The assay was performed as per the manufacturer's protocol.

**Anti-HBc IgM and IgG Detection:** ELISA kits from Dia.Pro. Diagnostics (Italy) were used for anti-HBc IgM and anti-HBc IgG determination. The assay was performed as per the manufacturer's protocol.

**HBV DNA Extraction:** Viral DNA was extracted from 200 µl of serum using the QIAamp Blood DNA Mini Kit (Qiagen, Germany) as per the kit protocol.

**HBV DNA Viral Load Quantification:** Quantitative determination of HBV DNA was performed using the Artus HBV RG PCR Kit (Qiagen). The master mix comprised 30 µl HBV RG/TM Master and 2 µl internal control per reaction, with 20 µl of extracted DNA template. Thermal cycling conditions included initial denaturation at 95°C

for 10 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds, annealing at 55°C for 30 seconds (with fluorescence acquisition in FAM and VIC channels), and extension at 72°C for 10 seconds. Detection of HBV DNA viral load correlated with HBV standards was done on the basis of amplification of signals into respective quencher dyes, along with the detection of internal controls.

**Classification of Infection Stage:** Patients were classified as having acute infection (HBsAg+, HBeAg+, anti-HBc IgM+, anti-HBc IgG−) or chronic infection (HBsAg+, HBeAg+/−, anti-HBc IgM−, anti-HBc IgG+) based on the serological marker profile.

## RESULTS:

**Seroprevalence of HBsAg:** Among the 1,500 screened patients, 51 (3.4%) tested positive for HBsAg by rapid card test, confirming intermediate endemicity in the Delhi NCR region according to WHO classification (2–7% = intermediate). The remaining 1,449 (96.6%) were non-reactive.[1]

**Table 1:** Distribution of Cases According to HBsAg Prevalence

| Cases | Number | Percentage |
|---|---|---|
| Positive | 51 | 3.4% |
| Negative | 1,449 | 96.6% |
| Total | 1,500 | 100% |

**Table 2:** Age and Sex Distribution of HBsAg-Positive Cases

| Age Group (Years) | Female | Male | Total |
|---|---|---|---|
| ≤21 | 2 | 2 | 4 |
| 22–31 | 2 | 3 | 5 |
| 32–41 | 7 | 3 | 10 |
| 42–51 | 2 | 9 | 11 |
| 52–61 | 3 | 6 | 9 |
| 62–71 | 3 | 3 | 6 |
| 72–81 | 2 | 4 | 6 |
| Total | 21 (41.2%) | 30 (58.8%) | 51 (100%) |

**Demographic Distribution:** Among the 51 HBsAg-positive patients, 30 (58.8%) were males and 21 (41.2%) were females, yielding a male-to-female ratio of 1.43:1. The highest prevalence was observed in the 42–51 year age group (11 cases, 21.6%), followed by the 32–41 year (10 cases, 19.6%) and 52–61 year (9 cases, 17.6%) age groups. Males predominated in the 42–51 years bracket (9 males vs 2 females), while females were more prevalent in the 32–41 years group (7 females vs 3 males).

**Table 3:** Distribution of Serological Markers Among HBsAg-Positive Cases

| Marker | Positive n (%) | Negative n (%) | Total |
|---|---|---|---|
| HBeAg | 48 (94.1%) | 3 (5.9%) | 51 |
| Anti-HBc IgM | 44 (86.3%) | 7 (13.7%) | 51 |
| Anti-HBc IgG | 7 (13.7%) | 44 (86.3%) | 51 |

HBeAg was positive in 48 out of 51 cases (94.1%), indicating a remarkably high rate of active viral replication in this cohort. Anti-HBc IgM was positive in 44 (86.3%) cases, suggesting predominantly acute or recent infection. Anti-HBc IgG was positive in only 7 (13.7%) cases.

**Table 4:** Distribution of HBV DNA Viral Load Levels

| HBV DNA Level (IU/mL) | Number | Percentage | p-value |
|---|---|---|---|
| <0.05 | 0 | 0% | <0.05 |
| >0.05–10 | 1 | 2.0% | |
| >10–2,000 | 15 | 30.6% | |
| >2,000–20,000 | 2 | 4.1% | |
| >20,000 | 31 | 63.3% | |
| Total | 49 | 100% | |

Viral load quantification was successfully performed on 49 of the 51 HBsAg-positive samples (2 samples were excluded because of not detecting viral load). The distribution of HBV DNA levels revealed that a majority of

patients (63.3%, 31/49) harboured very high viral loads exceeding 20,000 IU/mL, representing substantial transmission risk and meeting treatment initiation criteria. A further 30.6% (15/49) had moderate viral loads in the range of >10–2,000 IU/mL. Only 1 patient (2.0%) had a low viral load in the range of >0.05–10 IU/mL, and 2 patients (4.1%) had moderate-high viral loads in the range of >2,000–20,000 IU/mL.

**Table 5:** Distribution of Acute and Chronic HBV Infection

| Infection Type | Number | Percentage |
|---|---|---|
| Acute infection | 43 | 87.7% |
| Chronic infection | 6 | 12.3% |
| Total | 49 | 100% |

Based on the composite serological marker profile, 43 out of 49 patients (87.7%) were classified as having acute HBV infection, while 6 patients (12.3%) were classified as having chronic infection. The overwhelming predominance of acute infection is consistent with the high anti-HBc IgM positivity rate observed.

## DISCUSSION:

The present study provides a comprehensive assessment of HBsAg seroprevalence, serological marker profiles, and HBV DNA viral load distribution among patients at a tertiary care hospital in the Delhi NCR region. The findings provide key insights into the epidemiology and virology of HBV infection in this densely populated metropolitan area.

The HBsAg seroprevalence of 3.4% observed in our study places the Delhi NCR region within the intermediate endemicity zone as defined by the WHO (2–7%).[1] This finding broadly aligns with the nationally estimated HBsAg prevalence of approximately 3.7% in India.[2] Our prevalence figure is comparable to those reported from other hospital-based settings; Sood and Malvankar et. al. reported an HBsAg prevalence of 0.87% among hospital attendees in Jaipur.[8] Differences between hospital-based estimates may reflect variation in study populations and catchment areas.

The male predominance (58.8%) observed in our study is consistent with patterns reported in Indian HBV literature, although the mechanisms underlying sex differences are multifactorial.[3] The peak prevalence in the 42–51 years age group aligns with the economically productive age bracket, underscoring the socio-economic impact of HBV infection. The relatively high proportion of cases in the 32–51 years age range collectively (41.2%) suggests that some individuals may have acquired infection during childhood or adolescence, before hepatitis B vaccination was introduced as a national pilot component of the Universal Immunisation Programme in 2002.[9]

The remarkably high HBeAg positivity rate of 94.1% in our cohort is noteworthy and warrants careful interpretation. National and regional Indian data show considerably lower HBeAg positivity in many HBV-positive populations; a WHO India report summarized HBeAg positivity in the general population as ranging from 2.6% to 56%, with a mean of 24.2%, and reported regional variation.[10] The elevated HBeAg positivity in our study is likely attributable, at least in part, to the predominance of acute infections (87.7%) in the study population, during which HBeAg is commonly associated with active viral replication and higher infectivity.[5] This finding suggests that the majority of HBsAg-positive patients presenting to this tertiary care hospital were in an actively replicative phase.

The viral load distribution revealed that 63.3% of patients harboured very high HBV DNA levels (>20,000 IU/mL). This finding has direct clinical implications, as HBV DNA levels are an important component of treatment assessment, but treatment initiation should be individualized using HBV DNA together with HBeAg status, fibrosis/cirrhosis, and other clinical factors. [6,7] The classification of infection stage revealed that 87.7% of positive cases were acute infections, with only 12.3% chronic. This distribution differs from many Indian studies, which typically report a higher proportion of chronic carriers. The preponderance of acute cases in our study likely reflects the patient population of a tertiary care hospital, where symptomatic acute hepatitis patients are more likely to present and be tested, whereas chronic carriers may remain asymptomatic and undetected in the community.

The study has certain limitations that merit acknowledgment. First, as a hospital-based study, the seroprevalence figure may not be directly extrapolable to the general community population. Second, follow-up testing to determine the proportion of acute cases that progressed to chronic infection was beyond the scope of this cross-sectional design. Third, the relatively modest sample size of HBsAg-positive cases (n=51), while adequate for prevalence estimation, limits the statistical power of subgroup analyses.

## CONCLUSION:

The present study establishes that the Delhi NCR region demonstrates intermediate HBV endemicity with an HBsAg seroprevalence of 3.4%.[1] The remarkably high HBeAg positivity rate (94.1%) and the finding that 63.3% of patients harbour very high viral loads (>20,000 IU/mL) indicate widespread active viral replication and substantial transmission potential in this cohort.[5,6] Community-based seroprevalence surveys and longitudinal follow-up studies are recommended to further characterize the true burden and natural history of HBV infection in this population.

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