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Original Research Article

A Prospective Study on Role of Molecular Testing for Diagnosis of Chronic Hepatitis B Virus Infection in Patients Attending Tertiary Care Hospital at GMERS Medical College and Hospital, Sola, Ahmedabad, Gujarat

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Abstract:

Introduction: Hepatitis B virus (HBV) infection remains a major global public health problem and an important cause of chronic liver disease, cirrhosis, and hepatocellular carcinoma. Molecular testing has emerged as an essential tool for accurate diagnosis and monitoring of chronic HBV infection.

Materials and Methods: This prospective observational study was conducted at the Department of Microbiology, GMERS Medical College and Hospital, Sola, Ahmedabad. A total of 300 HBsAg-positive blood samples were analyzed. Serological testing and HBV DNA quantification by real-time polymerase chain reaction (PCR) were performed to evaluate the role of molecular testing in chronic HBV infection.

Results: Out of 300 HBsAg-positive samples, HBV DNA was detected in 226 cases (75.33%), while 73 cases (24.33%) were negative and 1 case (0.33%) was inconclusive. Male patients constituted 59.30% of positive cases, and the highest positivity was observed in the 21–40 years age group (46%). Viral load >20,000 IU/ml was observed in 38.05% of patients. Follow-up analysis demonstrated changes in viral load levels, highlighting the importance of serial molecular monitoring.

Conclusion: Real-time PCR is a valuable tool for detection and monitoring of chronic HBV infection and provides accurate assessment of viral replication and disease activity.

Keywords: Hepatitis B virus; HBV DNA; Real-time PCR; Molecular testing; Chronic hepatitis B; Viral load.

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Introduction

Hepatitis B virus (HBV) infection remains a major global public health problem and is one of the leading causes of chronic liver disease worldwide. [1] Viral hepatitis contributes significantly to morbidity and mortality due to complications such as chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. [2] According to global estimates, millions of individuals are living with chronic HBV infection, with a substantial disease burden observed in developing countries including India. [3] The prevalence of hepatitis B surface antigen (HBsAg) positivity in the Indian population varies considerably across different regions, indicating the persistent endemicity of the disease. [4]

Hepatitis B virus is a partially double-stranded DNA virus belonging to the family Hepadnaviridae. The infection may be transmitted through parenteral exposure, sexual contact, and perinatal transmission. [5] Clinical manifestations range from asymptomatic infection and acute hepatitis to chronic hepatitis, cirrhosis, and hepatocellular carcinoma. [6] A large proportion of infected individuals remain asymptomatic for prolonged periods, making early diagnosis difficult. [7] Chronic HBV infection is associated with continuous viral replication and progressive liver injury, which may eventually result in irreversible hepatic damage if not identified and treated at an early stage. [8]

Conventional serological markers such as HBsAg, HBeAg, and anti-HBc antibodies play an important role in the diagnosis of HBV infection; however, molecular methods have emerged as valuable tools for detecting viral replication and assessing disease activity. [9] Quantification of HBV DNA by molecular techniques such as real-time polymerase chain reaction (PCR) provides accurate information regarding viral load, infectivity, therapeutic response, and emergence of resistant strains. [10] Molecular testing has therefore become essential in the diagnosis, monitoring, and management of

chronic hepatitis B infection. [11] In view of the increasing burden of HBV infection and the growing importance of molecular diagnostics, the present study was undertaken to evaluate the role of molecular testing in the diagnosis of chronic hepatitis B virus infection in patients attending a tertiary care hospital in Ahmedabad, Gujarat.

Materials and Methods

This prospective observational study was conducted in the Department of Microbiology at GMERS Medical College and Hospital, Sola, Ahmedabad, Gujarat, after obtaining approval from the Institutional Ethics Committee. The study was carried out over a period of one and a half years. Patients attending the tertiary care hospital and clinically suspected to have chronic hepatitis B virus infection were included in the study. The aim of the study was to evaluate the role of molecular testing in the diagnosis of chronic hepatitis B virus infection.

Patients presenting with clinical features suggestive of hepatitis B infection and those advised for hepatitis B serological testing were considered for inclusion. Relevant demographic and clinical details were recorded in a predesigned proforma after obtaining informed consent. Blood samples were collected under strict aseptic precautions and transported to the microbiology laboratory for further processing. Serum was separated from collected blood samples and stored appropriately until testing was performed.

Initial screening for hepatitis B virus infection was carried out using serological markers. Detection of hepatitis B surface antigen (HBsAg) was performed as the primary screening test. Additional serological markers including HBeAg, anti-HBc, and anti-HBs were assessed wherever indicated for evaluation of infection status and disease activity. Liver function parameters and relevant biochemical investigations were also considered during assessment of patients with chronic HBV infection. Molecular testing was performed for detection and quantification of HBV DNA using real-time polymerase chain reaction (PCR) techniques. HBV DNA estimation was utilized to assess viral replication, infectivity, and disease progression. The molecular assay also helped in differentiating active chronic hepatitis B infection from inactive carrier states and was useful in monitoring response to antiviral therapy. Patients were further categorized based on serological profile, HBV DNA levels, and clinical findings. The obtained data were compiled and analyzed to determine the positivity rate of chronic hepatitis B virus infection and to evaluate the significance of molecular testing in diagnosis and management of HBV infection.

Results

Out of 300 HBsAg-positive samples tested by real-time PCR, HBV DNA was detected in 226 patients (75.33%), while 73 patients (24.33%) were negative for HBV DNA. One sample (0.33%) showed an inconclusive result. These findings indicate the importance of molecular testing in confirming active HBV infection among seropositive individuals.

Table 1: Positivity Rate of Chronic Hepatitis B Virus Infection by Real-Time PCR (n=300)

Result	Number of Cases	Percentage
HBV DNA detected	226	75.33%
HBV DNA not detected	73	24.33%
Inconclusive	1	0.33%
Total	300	100%

Among the 226 HBV DNA-positive patients, males constituted the majority with 134 cases (59.30%), followed by females with 90 cases (39.82%) and transgender patients with 2 cases (0.88%). Age-wise distribution showed that the highest number of positive cases belonged to the 21–40 years age group (46.00%), followed by 41–60 years (31.00%), suggesting higher prevalence among the economically productive age group.

Table 2: Demographic Distribution of HBV DNA Positive Patients (n=226)

Parameter	Number of Patients	Percentage
Gender Distribution		
Male	134	59.30%
Female	90	39.82%
Transgender	2	0.88%
Age Group Distribution		
0–20 years	19	8.40%
21–40 years	104	46.00%
41–60 years	70	31.00%
>60 years	33	14.60%

Analysis of HBV viral load demonstrated that 86 patients (38.05%) had HBV DNA levels greater than 20,000 IU/ml, while 81 patients (35.85%) had viral loads below 2,000 IU/ml. Moderate viral load levels between 2,000–20,000 IU/ml were observed in 59 patients (26.10%).

Table 3: Distribution of HBV DNA Viral Load Among Positive Cases (n=226)

HBV DNA Copies	Number of Patients	Percentage
<2000 IU/ml	81	35.85%
2000–20000 IU/ml	59	26.10%
>20000 IU/ml	86	38.05%
Total	226	100%

Follow-up evaluation was available for 11 patients to assess changes in viral load over time. Some patients demonstrated reduction or clearance of HBV DNA during follow-up, whereas a few initially negative patients became HBV DNA positive later.

Table 4: Follow-Up Viral Load Analysis in Chronic HBV Patients

Patient No.	Initial HBV DNA Status	Initial Viral Load (IU/ml)	Follow-Up HBV DNA Status	Follow-Up Viral Load (IU/ml)
4	Detected	39,300	Detected	99,600
62	Detected	3660000000	Detected	392400000
94	Detected	299.4	Not detected	0
129	Detected	272400000	Detected	3051000
158	Not detected	0	Not detected	0
165	Detected	114	Not detected	0
197	Detected	128	Detected	121
199	Detected	3350000	Detected	10260
217	Not detected	0	Detected	9450
226	Detected	10400	Detected	5640
261	Not detected	0	Detected	29.07

The overall study findings revealed a high positivity rate of HBV DNA among HBsAg-positive individuals, with male predominance and maximum cases occurring in the 21–40 years age group. Molecular testing by real-time PCR proved useful for detection of active viral replication, assessment of viral load, and monitoring of chronic HBV infection during follow-up.

Discussion

The present study demonstrated that among 300 HBsAg-positive patients, HBV DNA was detected in 226 cases (75.33%) by real-time PCR, highlighting the important role of molecular testing in confirmation of active hepatitis B virus infection. Similar findings were reported by Abazuh et al. [12] (2023) from Nigeria, who observed a significantly higher detection rate of HBV infection by PCR-based molecular assay (18.2%) compared to routine rapid serological testing (5.8%), emphasizing the superior sensitivity of molecular techniques in detecting active viral replication and occult infection. Likewise, Lee and Kim [13] (2021) stated that molecular assays such as real-time PCR are essential for accurate diagnosis, monitoring of viral replication, and assessment of treatment response in chronic HBV infection.

The findings of the present study therefore support the growing evidence that reliance only on serological markers may fail to identify all actively infected individuals and that molecular testing provides better diagnostic accuracy for chronic hepatitis B infection. In the present study, male predominance was observed among HBV DNA-positive patients, with males accounting for 59.30% of cases, while females constituted 39.82% of positive cases. Similar observations were noted in several previous studies. Koech et al. [14] (2023) reported that males accounted for 60% of HBV infections among outpatient attendees in Kenya. Anka et al. [15] also observed a higher prevalence of HBV infection among male blood donors in Nigeria. Similarly, Agarwal et al. [16] (2018) found higher seroprevalence of HBV among males in a tertiary care hospital population in Uttar Pradesh. The higher prevalence among males may be related to increased occupational exposure, behavioral risk factors, higher mobility, and greater likelihood of exposure to unsafe injections, blood products, and unprotected sexual practices. The consistency of male predominance across different geographic regions supports the epidemiological trend observed in our study population.

Age-wise analysis in the current study showed that the majority of HBV DNA-positive patients belonged to the 21–40 years age group (46%), followed by the 41–60 years age group (31%). These findings are comparable with those reported

by Abazuh et al. [12] (2023), who observed higher relative risk and odds ratios among individuals aged 21–30 years and 31–40 years. Similarly, Koech et al. [14] (2023) reported that HBV infection was most prevalent among individuals aged 31–40 years, while Anka et al. [15] found the highest prevalence of HBV serological markers among participants aged 28–37 years. The predominance of HBV infection in younger and middle-aged adults may be attributed to increased social activity, occupational exposure, sexual transmission, migration, and greater interaction with healthcare systems. Since this age group represents the economically productive population, chronic HBV infection can lead to substantial socioeconomic and healthcare burdens if early diagnosis and treatment are not ensured.

The present study also evaluated HBV viral load distribution among positive patients and found that 38.05% of patients had viral loads greater than 20,000 IU/ml, indicating active viral replication and increased infectivity. Patients with lower viral loads (<2000 IU/ml) accounted for 35.85% of cases and may represent inactive carrier states. Similar observations were reported by Belayneh et al. [17] (2025), who found increased HBV viral load and higher HBeAg positivity among HBsAg-positive patients with coexisting Helicobacter pylori infection, suggesting that associated infections may influence HBV replication activity. Anka et al. [15] also demonstrated that most HBV-infected blood donors had HBV DNA levels below 2000 IU/ml, corresponding to inactive carrier phases, while a subset showed higher viral loads requiring close monitoring and antiviral therapy.

These findings collectively indicate that viral load estimation by real-time PCR is crucial not only for diagnosis but also for assessment of infectivity, disease progression, and therapeutic decision-making in chronic hepatitis B infection.

Follow-up analysis performed in the present study demonstrated dynamic changes in HBV viral load over time, with some patients showing reduction or complete disappearance of HBV DNA, while a few initially negative patients later became HBV DNA positive. These observations highlight the importance of serial molecular monitoring in chronic HBV infection. Similar conclusions were emphasized by Lee and Kim [13] (2021), who stated that molecular monitoring plays an important role in evaluating treatment response and identifying ongoing viral replication. Ramya et al. [18] (2025) also demonstrated the clinical importance of HBV DNA testing in detecting occult hepatitis B infection among HBsAg-negative chronic liver disease patients, showing that molecular assays can identify hidden infections missed by routine serology.

Furthermore, Shrestha et al. [19] (2020) from Nepal highlighted the importance of combining serological and molecular analysis for characterization of chronic HBV infection and detection of different genotypes. Thus, the findings of the present study reinforce the clinical utility of molecular testing as an indispensable tool for diagnosis, monitoring, prognostic evaluation, and management of chronic hepatitis B virus infection.

The study was conducted at a single tertiary care center with a relatively limited sample size, which may restrict generalization of the findings to the wider population. Long-term follow-up of all patients could not be performed, and detailed genotypic analysis of HBV strains was not included in the study.

Conclusion

Molecular testing by real-time PCR plays an important role in the diagnosis and monitoring of chronic hepatitis B virus infection. HBV DNA detection provides accurate assessment of active viral replication, infectivity, and treatment response, making it a valuable adjunct to conventional serological testing in the management of chronic HBV infection.

Bibliography

1. Lavanchy D. Chronic viral hepatitis as a public health issue in the world. Best Pract Res Clin Gastroenterol. 2008;22(6):991–1008.
2. Shen C, Jiang X, Li M, Luo Y. Hepatitis virus and hepatocellular carcinoma: recent advances. Cancers. 2023;15(2):533.
3. Premkumar M, Chawla YK. Chronic hepatitis B: challenges and successes in India. Clin Liver Dis. 2021;18(3):111–6.
4. Hsu YC, Huang DQ, Nguyen MH. Global burden of hepatitis B virus: current status, missed opportunities and a call for action. Nat Rev Gastroenterol Hepatol. 2023;20(8):524–37.
5. Mylonas I. Hepatitis B and D. In: Sexually Transmitted Diseases: A Guide for Gynecologists and Obstetricians. Springer; 2025. p. 445–70.
6. Di Bisceglie AM. Epidemiology and clinical presentation of hepatocellular carcinoma. J Vasc Interv Radiol. 2002;13(9):S169–71.
7. Meyerowitz EA, Richterman A, Bogoch II, Low N, Cevik M. Towards an accurate and systematic characterisation of persistently asymptomatic infection with SARS-CoV-2. Lancet Infect Dis. 2021;21(6):e163–9.
8. Shi YH, Shi CH. Molecular characteristics and stages of chronic hepatitis B virus infection. World J Gastroenterol WJG. 2009;15(25):3099.
9. Kao JH. Diagnosis of hepatitis B virus infection through serological and virological mark-

ers. Expert Rev Gastroenterol Hepatol. 2008;2(4):553–62.
10. Hatzakis A, Magiorkinis E, Haida C. HBV virological assessment. J Hepatol. 2006;44: S71–6.
11. Jardi R, Rodriguez F, Buti M, Costa X, Cotrina M, Valdes A, et al. Quantitative detection of hepatitis B virus DNA in serum by a new rapid real-time fluorescence PCR assay. J Viral Hepat. 2001;8(6):465–71.
12. Abazuh UD, Adebayo OH, Losh NB, Adeyemo DA, Bello OK, Oyediran OS, et al. Detection and prevalence of HBsAg and HBV DNA among visiting patients attending health facilities in Nigeria. Sci Afr. 2023;20:e01702.
13. Lee J, Kim H. Current laboratory tests for diagnosis of hepatitis B virus infection. Int J Clin Pract. 2021;75(12):e14812.
14. Koech SJ. Prevalence and Genotypic Characterization of Hepatitis B Virus among Outpatient Attendees at Moi Teaching and Referral Hospital, Eldoret. 2023.
15. Anka A, Musa B, Ella E, Hassan A, Manko M, Umar K, et al. Evaluation of hepatitis B virus infection serological markers and viral load profile of potential blood donors attending Ahmadu Bello University Teaching Hospital, Zaria-Nigeria. Sokoto J Med Lab Sci. 2024;9(2):87–97.
16. Agarwal L, Singh AK, Agarwal A, Singh RP. Incidental detection of hepatitis B and C viruses and their coinfection in a hospital-based general population in tertiary care hospital of Uttar Pradesh. J Fam Med Prim Care. 2018;7(1):157–61.
17. Belayneh M, Tegegne BA, Tizazu AM, Yeshinew AG, Lemma M, Abay Z, et al. Serological and virological characterization of hepatitis B surface antigen-positive patients with or without Helicobacter pylori co-infection at the university of Gondar comprehensive specialized hospital, Northwest Ethiopia. BMC Infect Dis. 2025;25(1):1273.
18. Ramya S, Nair S, Krishnamurthy V, Kanungo R. Occult hepatitis B virus infection among chronic liver disease patients: Insights from a tertiary care hospital. Indian J Med Microbiol. 2025;56:100903.
19. Shrestha S, Mahatara S, Pun SB, Shrestha M, Napit R, Manandhar KD. Serological markers and molecular analysis of hepatitis B infection in a tertiary care hospital at Kathmandu, Nepal. Indian J Gastroenterol. 2020;39(4):354–61.