Content available at: https://www.ipinnovative.com/open-access-journals

IP International Journal of Medical Microbiology and Tropical Diseases

Journal homepage: https://www.ijmmtd.org/

Original Research Article

Prevalence of HBsAg infection and treatment predictors in HBsAg-positive patients at tertiary care center

Vaishali Bhupatbhai Bhadreshwara[1]*, Hiral M Gadhavi[1], Hitesh K Shingala[1]

[1]Dept. of Microbiology, Shri M.P. Shah Government Medical College, Jamnagar, Gujarat, India

Abstract

Background: HBV infection is a global public health challenge, contributing significantly to liver-related morbidity and mortality. This study, conducted at a tertiary care center, aimed to decide the prevalence of HBV using ELISA test, assess Hepatitis B virus viral load in HBsAg-positive patients, and evaluate predictors of treatment response in those receiving antiviral therapy.

Materials and Methods: This study included 42000 samples from suspected cases of viral Hepatitis for HbsAg antigen testing. The samples were checked for presence of HBsAg antigen by 3rd generation ELISA. The samples positive for HBV infection were then tested for HBV DNA by quantitative RT-PCR test.

Results: Total 42,000 samples of patients were taken for testing for HBsAg Antigens by Enzyme-Linked Immunosorbent Assay, 620 (1.47%) were seropositive. Out of 620 samples, 122 samples had taken testing for the presence of HBV DNA level by real time RT-PCR. Out of 122 samples, 102 samples were detected positive for HBV DNA and 20 were detected negative by PCR. Out of 102 HBV DNA positive samples, 44 samples had HBV DNA level <20,000 IU/ml and 58 samples had HBV DNA level >20,000 IU/ml.

Conclusion: Hepatitis B is continued to be serious global threat with long term complication. In developing countries HBV is a major cause of mortality and morbidity. HBV RT-PCR has high sensitivity & specificity for detecting active infection and monitoring response to treatment.

Keywords: Hepatitis B, HBsAg, ELISA, HBV-DNA, treatment predictors, ALT level, cirrhosis, hepatocellular carcinoma.

Received: 21-05-2025; **Accepted:** 01-07-2025; **Available Online**: 04-09-2025

1. Introduction

Hepatitis B virus is a major cause of viral Hepatitis worldwide, with profound implications for liver health. The World Health Organization (WHO) have given estimation of people with chronic HBV infection, in 2022, 254 million people had chronic infection of hepatitis B virus, which leads to 1.1 million deaths, primarily due to cirrhosis and HCC.[1] HBV is transmitted through perinatal, parenteral, and sexual routes, with significant regional variation in prevalence and transmission patterns. In highly endemic areas, such as parts of Africa and Asia, perinatal transmission dominates, while in lower-endemicity regions, sexual and parenteral exposures are more common.[2]

India, classified as an intermediate endemicity zone, harbors 10-15% of the global HBV carrier pool, with an estimated 40 million chronic carriers.[3] The range of prevalence of HBsAg antigen infection in the general population is 1.1% to 12.2%, averaging 3-4%.[3]

Chronic Hepatitis B virus infection resulting in 40-50% of HCC & 20-30% of cirrhosis cases in the country, posing a substantial public health burden [3]. While acute HBV infection is often self- limiting, chronic infection can lead to severe hepatic complications, including fulminant hepatitis, cirrhosis, and HCC.[4]

The advent of serological testing, notably ELISA, has revolutionized HBV diagnosis, enabling early detection of HBsAg as a marker of infection. Quantitative assays, such

*Corresponding author: Vaishali Bhupatbhai Bhadreshwara
Email: bhadreshwara.vaishali@gmail.com

https://doi.org/10.18231/j.ijmmtd.v.11.i.3.19

as RT-PCR, further assess HBV DNA, guiding treatment decisions and monitoring prognosis.[5] This study, conducted at a tertiary care center, the main aim of this study is to estimate seroprevalence of HBV infection, quantify viral load in HBsAg- positive patients, and identify predictors of treatment efficacy, contributing to the evidence base for managing HBV in resource-limited settings.

2. Materials and Methods

This retrospective and prospective Study had been conducted in Department of Microbiology for the period of 12 months from January 2023 to December 2023. The blood samples for study had been received from various departments of hospital and then tested for presence of HbsAg antigen.

Total 42000 samples were taken for study from suspected cases of viral Hepatitis for HbsAg antigen testing. 3ml to 5ml blood had been withdrawn by aseptic venepuncture method & then it transferred to plain Tube for HBsAg antigen testing and received in laboratory. The tube was then rotated at 1500rpm in centrifuge for 10 minutes to separate the serum. All samples were tested for HBsAg antigen by 3rd generation ELISA. All HBsAg positive patients are further referred by their physician to treatment center, then do registration under NVHCP guideline for investigation & HBV Viral Load testing, then according to NVHCP guideline treatment was started. All HBV positive samples were taken for testing of HBV DNA by quantitative RT-PCR. For testing, 10ML blood was taken for plasma in EDTA Tube and then received at Laboratory.

2.1. Procedure for HBsAg antigen ELISA

2.1.1. Principle

Hepalisa HBsAg is a 3rd generation ELISA & is intended to be used for detection of Hepatitis B surface antigen in Human serum/plasma.

2.2. Molecular study

All HBV Seropositive samples were confirmed by RT -PCR.

2.2.1. Procedure for HBV PCR

HBV DNA detection was done by automated extraction method using automated system and by manual extraction method. For automated extraction, fluorescently labelled TAQMAN probes were used to detect the amplicon in APPLIED BIOSYSTEMS -7500 RT PCR system. Manual extraction method was done by GSure Viral DNA Isolation kit.

2.2.2. PCR Kit

TRUPCR HBV Viral Load Kit
Number of reactions: 96

The limit of detection of TRUPCR HBV viral load kit for the detection of HBV in EDTA plasma is 2.5 IU/ML. The range of the TRUPCR HBV Viral Load Kit for quantification of HBV viral load in EDTA plasma is 2.5-10,000,000 IU/ml.

To decide the viral load of the sample, use the following formula:

$$Viral\ load\ (sample)\ IU/ml = \frac{Volume\ (Elute)\ [\mu l]\ x\ Concentration\ of\ sample\ [IU/\mu l]}{Sample\ input\ [ml]}$$

After that, HBV DNA positive patients treated with Anti-viral drugs for 12 weeks. Then after 3 months of completion of treatment, we have to see the effectiveness of treatment by follow up HBV DNA detection in blood and detection of ALT level.

3. Results

Total 42,000 Samples were received and tested in the department of microbiology for testing of HBsAg Antigen ELISA during the study period of 12 months from January 2023 to December 2023.

Among 42,000 patients goes under study group, 22598 (53.80%) were males and 19402 (46.20%) were females.(**Table 1**)

Table 1: Gender-wise distribution of study population

Gender	No. of patients	Percentage
Male	22598	53.80%
Female	19402	46.20%
Total	42000	100%

ELISA was used for detection of HBsAg Antigens. Out of 42,000 samples those tested for HBsAg Antigens by serological test, 620 (1.47%) were positive which is seen in following **Table 2**.

Table 2: Sero-prevalence of hepatitis B infection among patients in a tertiary care hospital

Total No. of samples received	Total No. of positive samples	Percentage of total No. of positive samples
42000	620	1.47%

The analysis according to age in present study showed that high sero-positivity was in patients in the age group of 21-30 Years (31.77%) then in age group 31-40 Years (17.58%) and 51-60 Years (15.48%). Lowest prevalence observed in patients in age group greater than 80 years (0.43%).(**Table 3**)

Table 3: Age-wise distribution of HBsAg positive cases

Age groups	No. of cases	Percentage
0-20yrs	53	8.55%
21-30yrs	197	31.77%
31-40yrs	109	17.58%
41-50yrs	86	13.87%
51-60yrs	96	15.48%
61-70yrs	50	8.06%
71-80yrs	26	4.19%
>80yrs	3	0.48%
Total	620	100%

Among the 620 HBsAg positive patients, 326 (52.58%) and 294 (47.42%) were males and females respectively which showed in **Figure 1**.

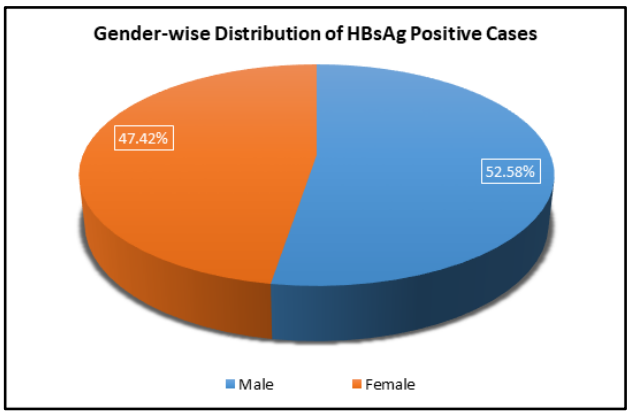

Figure 1: Gender-wise distribution of HBsAg positive cases

In this study, hemodialysis was probable risk factor for HBV transmission as hemodialysis in 200 Cases (32.26%), Surgery and Blood transfusion in 133 Cases (21.45%), HIV Co-Infection in 61 cases (9.84%), and presence of Liver Disease in 34 cases (5.48%). In other 192(30.97%). HBsAg positive cases had no risk factor.(**Table 4**)

Table 4: Distribution based on probable history of exposure to HBV infection.

Risk factor	No. of HBsAg Positive Cases
Hemodialysis	200(32.26%)
Surgery and Blood transfusion	133(21.45%)
HIV Co-Infection	61(9.84%)
Presence of liver disease	34(5.48%)
Unknown	192(30.97%)
Total	620(100%)

Liver enzymes in HBV related chronic liver disease are may be fluctuating or normal. In present study, Alanine aminotransferase was elevated in about 38.39% HBsAg positives. **Figure 2**

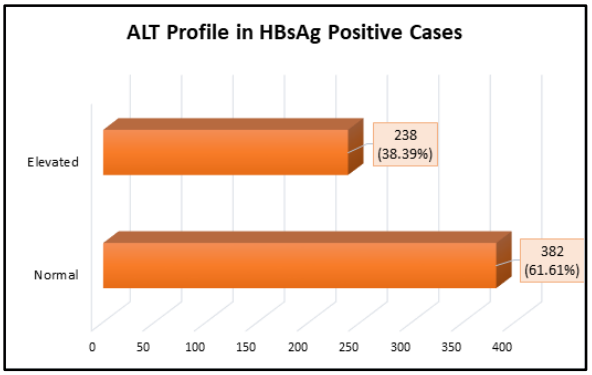

Figure 2: ALT profile in HBsAg positive cases (n=620)

Out of 620 samples, 122 samples were tested for presence of viral load by real time RT-PCR as other 498 HBsAg positive patients were in acute stage and these were not tested for the presence of HBV DNA.

Out of 122 samples taken for testing for presence of HBV DNA by real time RT-PCR, 102 were tested positive for HBV DNA and 20 were detected negative by PCR.(**Table 5**)

Table 5: Result of HBV PCR in HBsAg positive cases

HBV PCR	No. of cases	Percentage
Positive	102	83.60%
Negative	20	16.40%
Total	122	100%

Out of 102 HBV DNA positive samples, 44 samples had HBV DNA level <20,000 IU/ml & 58 samples had HBV DNA level >20,000 IU/ml. **Figure 3**

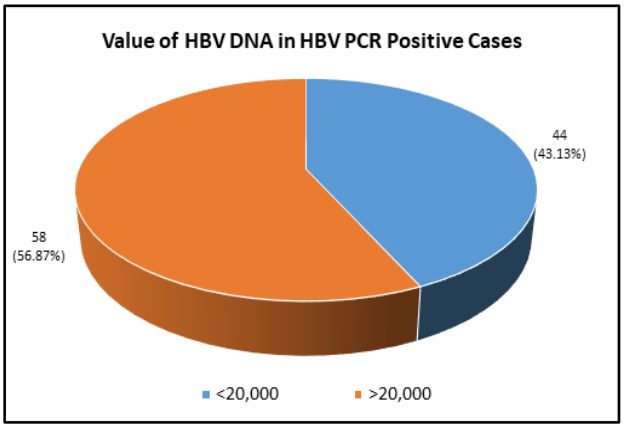

Figure 3: Value of HBV DNA in HBV PCR positive cases

Out of 58 patients had HBV DNA level >20,000 IU/ml, 37 patients started treatment for HBV infection according to NVHCP guideline, 2 patients died before starting treatment, 6 patients were medically not eligible for treatment, 4 patients were not come for treatment after registration, 2 patients were tested negative by PCR after treatment and 7 patients were not come for registration for treatment.

Out of 37 patients started treatment, 23 patients discontinued the treatment after 12 weeks whereas 14 patients continued the treatment.(**Table 6**)

Table 6: Treatment status of patients after 12 weeks of starting of the treatment

Treatment status of patients	No. of patients	Percentage
Continue treatment	14	37.84%
Discontinue treatment	23	62.16%
Total	37	100%

4. Discussion

HBV Infection is a very serious threat to Health Care system because it can cause Clinical conditions ranging like acute infection to Chronic hepatitis and Hepatocellular carcinoma (HCC). Depending on standards of living, prevalence of HBV infection is varied in different countries. In developed & developing Countries it differs in age group and also in mode of transmission; Countries or areas with high standards of living has high prevalence & countries or areas with low socioeconomic levels has low prevalence. Currently India is in an Intermediate endemicity zone.

In this study, 42,000 patients were included as study Population. The serological test like ELISA was done for detecting HBsAg Antigen. Determination of HBV DNA after the completion of treatment for HBV infection was done by molecular assay.

Table 7: Comparison of sero-prevalence of HBV infection

S.No.	Study	Study region	Sero prevalence rate
1	Bharat Singh et al[12] 2021	Central India	2.05%
2	Prity P. Narwade et al[7] 2019	West India	1.90%
3	Patil et al[8] 2016	West India	2.25%
4	Manoj Kumar et al[10] 2019	North India	4.80%
5	Trupti B. Naik et al[9] 2018	South India	0.56%
6	Present study	West India	1.47%

In the present study, 42,000 patients were screened for HBsAg Antigen. Out of these 620 (1.47%) were tested positive. So, the sero- prevalence of HBV infection is 1.47%.

The similar to study done by Bharat Singh et al[6] (2.05%), Prity P. Narwade et al[7] (1.90%) and Patil et al[8] (2.25%). Lower sero positivity was reported from Trupti B. Naik et al[9] (0.56%), while other Study done by Manoj Kumar et al[10] and it Showed the higher Prevalence (4.80%) as compared to present Study.(**Table 7**)

This Difference in HBV seroprevalence, cannot be completely explained. These differences might be explained by various reasons like Different type of population studied, different geographical region, socioeconomic status of patients, health factors, genetic factors, awareness of routes of HBV transmission, efforts made to implement Universal Precautions by Health professionals and compulsory screening of hepatitis B virus prior to blood donation and any surgical procedure.[11]

Table 8: Comparison of age group distribution among positive HBsAg samples

S.No.	Study	Most commonly affected age group	Percentage
1	Parimal H.Patel et al[12] 2016	21-30years	31.54%
2	Bharat Singh et al[6] 2021	21-30years	29.60%
3	Present study	21-30years	31.77%

Among this study, patients in Age group of 21-30 Years (31.77%) were commonly affected. This finding correlates to study performed by Parimal H.Patel et al,[12] which shows similar result that prevalence was seen more in Age groups 21-30 Years. One more Study, which is performed by Bharat Singh et al[6] which also shows similar result.(**Table 8**)

More prevalence among 21-30 years because this range of age group is Economically and Sexually active age group. So, more chances of Horizontal transmission, which might be due to unsafe Injection practices, Intravenous drug abuse and unsafe Sexual practices.

Regarding positivity among hepatitis B reactive patients, this Study has shown that there were 326 (52.58%) Males and 294 (47.42%) Females suggests predominance of Male gender.

Similar result was showed by Prity P.Narwade et al[7] It showed significantly higher prevalence (55.28%) of Male HBsAg positive Patients. Similar result has shown by Manoj Kumar et al[10] in their study.

Hemodialysis was major risk factor which is observed in 32% than blood transfusion in 21% and HIV Co-infection in 10%.

Hemodialysis history was seen in 32% positive samples. This result is similar with study by Wajeeha Elahi et al[13] (2020) which showed 67% & Dimple Raina et al[14] (2022) which was 12%.

This might be explained by the fact that HBV infection is high among patients who are on hemodialysis as a result of cross contamination from dialysis circuit. For reduction of this transmission strict infection practises are needed.

In patients in which no risk factor were identified for HBV infection, cause may be sexual transmission or any subcutaneous procedure like tattooing that patient does not know about exposure.

In present study liver enzyme level (ALT) was higher in HBsAg positive patients. This is due to liver parameter is fluctuating in HBV infection and sometimes normal. This study is similar with Ajay Kumar et al.[15]

Out of 620 samples, 122 samples were taken for testing to determine presence of HBV DNA by real time RT-PCR as other 498 HBsAg positive patients were in acute stage, clinicians have not sent samples for testing so, these samples were not taken for testing.

Among 122 HBsAg ELISA positive cases tested for HBV DNA, 102(83.60%) were positive for HBV DNA.

The result is similar with study done by Marcelo Eidi Nita et al[16] that show HBV DNA detection rate of 78.3%. Another similar study was done by Iregbu KC et al[17] which show HBV DNA detection rate of 76.1%. This variation may be because of intermittent viremia or spontaneous resolution of infection.[11]

Individuals who had taken vaccine for Hepatitis B may give a transient positive result for HBsAg infection because of its Presence in the Vaccine.[18] Hence, confirmation should be done by testing for HBV DNA level.

Out of 102 Positive HBV DNA patients, 58 patients had HBV DNA level >20,000 IU/ml, so they were eligible for starting the treatment but only 37 patients started treatment. The reasons for not starting treatment were, patient not eligible for treatment, died before starting the treatment and not come for treatment. After starting anti-viral therapy in HBV PCR positive patients, after 12 Weeks of completion of treatment during monitoring of response, 14 patients continued treatment while 23 patients discontinued treatment.

5. Conclusion

Hepatitis B continues to be serious global threat with long term complication. In developing countries HBV is a major cause of mortality & morbidity. Hepatitis B virus has wide geographic variations with multiple genotypes and subtypes. India stands in the intermediate zone in prevalence of HBV infection. Hence present study is about that Hepatitis B Virus infection is still a major public health problem.

In this study, prevalence of HBV infection is more in chronic liver disease patients than general population. So, preventive strategies like vaccination, prompt treatment, early case detection, and the most importantly, the general awareness in general population can reduce the burden of infection.

3rd generation ELISA is very useful and cost-effective screening test for serological diagnosis of HBV infection. It is affordable, reliable and easy to use with good clinical outcome. Hepatitis B viral load by RT-PCR in HBsAg positive patients gives the actual amount of viral replication in blood, because it is very Sensitive and Specific Method to determine active Infection and also monitoring response to therapy.

As Liver enzyme (ALT) level is good indicator of liver damage in chronic HBV patients, but HBV DNA by RT-PCR is more important indicator for starting treatment and for monitoring the response to therapy. In places where HBV viral load testing is not possible, treatment might be decided based on persistently abnormal ALT levels, but other causes of persistently high ALT levels such as dyslipidaemia, Impaired glucose tolerance and Fatty liver should be excluded.

6. Authors' Contributions

All authors listed have made a substantial and direct contributions to work and approved it for publication.

7. Data Availability

All datasets generated and analysed during this study are included in the manuscript.

8. Source of Funding

None.

9. Conflict of Interest

None.

10. Acknowledgements

None.

References

1. World Health Organization. Global progress report on HIV, viral hepatitis and sexually transmitted infections, 2021. Available at: https://www.who.int/publications/i/item/9789240027077
2. Villa DF, Maria-Cristina N. Vertical Transmission of Hepatitis B Virus—An Update. *Microorganisms*. 2023;11(5):1140.
3. Puri P. Tackling the hepatitis B disease burden in India. *J Clin Exp Hepatol*. 2014;4(4):312–9.
4. Trépo C, Chan HL, Lok A. Hepatitis B virus infection. *Lancet*. 2014;384(9959):2053–63.
5. Pawlotsky JM. Molecular diagnosis of viral hepatitis. *Gastroenterology*. 2002;122(5):1554–68.
6. Singh B, Jayant S, Dehariya R, Poddar A, Bajpai T. Seroprevalence of Hepatitis B Virus infection in patients visiting a tertiary care hospital in Central India. *Int J Health Clin Res*. 2021;4(14):130–2.
7. Narvade PP, More SR, Kandle SK, Rathod VS, Emekar SM. Seroprevalence of Hepatitis B Surface Antigen (HBsAg) among Patients Attending a tertiary Care Hospital. *Int J Curr Microbiol App Sci*. 2019;8(3):1014–8.
8. Patil SR, Ghorpade M, Patil SS, Pawar S, Mohite S. Seroprevalence of Hepatitis-B Surface antigen among the patients reporting at tertiary care Hospital from India. *Bangladesh J Med Sci*. 2016;15(3)::455–9.

9. Naik TB, Satish JV, Wadekar MD. Seroprevalence of Hepatitis B Surface Antigen (HBsAg) among Patients Attending a tertiary Care Hospital at Chamarajanagar, Karnataka, India. *Int J Curr Microbiol App Sci*. 2018;7(1):1279–84.
10. Kumar M, Verma RK, Nirjhar S, Singh M. Seroprevalence of Hepatitis B at tertiary Care Hopital. *Ann Int Med Den Res*. 2019;5(3):5–8
11. Mandell GL, Bennett JE, Churchill DR. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. Edition 9th. Livingstone. 2020;2040–70.
12. Patel PH, Nerurkar AB, Patel MR. Seroprevalence of Hepatitis B Surface Antigen in patients attending a tertiary care hospital Valsad, South Gujarat, India. *Int J Med Microbiol Trop Dis*. 2016;2(3):103–6.
13. Elahi W, Syed AZ, Nasim F, Anwar A, Hashmi AA. Hepatitis B and C Infections in Patients with Prolonged Hemodialysis Secondary to Chronic Renal Failure. *Cureus*. 2020;12(10):e10905.
14. Raina D, Rawat N, Ajay K. Pandita. Prevalence of Hepatitis B and Hepatitis C in patients Undergoing hemodialysisi at teaching hospital in Uttarakhand. *J Family Med Prim Care*. 2022;11(4):1348–53.
15. Kumar A, Pant S, Narang S. Significance of alanine aminotransferase testing in diagnosis of acute and chronic HBV infection. *Asian Pac J Cancer Prev*. 2009;10(6):1171–2.
16. Nita ME, Gaburo N, Cheinquer H, L'Italien G, Affonso de Araujo ES, Mantilla P, et al. Patterns of viral load in chronic hepatitis B patients in Brazil and their association with ALT levels and HBeAg status. *Ann Hepatol*. 2009;8(4):339–45.
17. Iregbu KC, Nwajiobi-Princewill PI. Viral load pattern among hepatitis B surface antigen-positive patients: Laboratory perspective and implications for therapy. *Ann Med Health Sci Res*. 2016;6(2):95–9
18. Anjum Q. False positive Hepatitis B Surface Antigen due to recent vaccination. *Int J Health Sci (Qassim)*. 2014;8(2):189–93.

Cite this article: Bhadreshwara VB, Gadhavi HM, Shingala HK. Prevalence of HBsAg infection and treatment predictors in HBsAg-positive patients at tertiary care center. *IP Int J Med Microbiol Trop Dis*. 2025;11(3):352-357.