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Original Research Article

ASSESSMENT OF HBV DNA VIRAL LOAD IN SEROLOGICALLY POSITIVE INDIVIDUALS – A CROSS SECTIONAL STUDY

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ABSTRACT

Background: Hepatitis B is considered the most prevalent and consequential form of viral hepatitis, with the potential to progress to a chronic state and result in complications such as cirrhosis and hepatocellular carcinoma. **Objective:** The objective of this investigation was to evaluate serological indicators HBsAg, HBeAg, and anti-HBe in cases of Hepatitis B virus infection and contrast them with viral load measured through RT PCR analysis.

Materials and Methods: A cross-sectional study was undertaken with 81 patients who had tested positive for HBsAg for a minimum of 6 months, while attending the Outpatient Department and Inpatient Department of Gastroenterology and General Medicine, GSL Medical College. Rajahmundry.

Results: Out of 81 HBsAg positive patients, HBV-DNA was detected in 54 patients. Among these, 19 (35.2%) patients were HBeAg positive, 24 (44.4%) patients were anti-HBe positive and 11 (20.4%) were negative for both HBeAg and anti-HBe. Age limit of patients was up to 65 years. HBV-DNA positive patients showed male predominance; 41 (76%) patients were male and 13 (24%) patients were female. Mean age of the patients was 36±12 years. Among 81 HBsAg positive patients, twenty four were receiving antiviral therapy. Out of them, HBV-DNA was decreased among 11 patients and could not be detected among 13 patients. **Discussion:** HBV is significant to medicine and public health not only as the cause of acute liver disease, but also as the source of chronic, persistent infections that can lead to the death of infected individuals from cirrhosis and liver cancer. Chronically infected individuals act as the virus reservoir in the population. In the majority of cases, the original infection is asymptomatic and disappears due to an efficient cell-mediated immune response.

Conclusion: Real time PCR method of detection of HBV-DNA is very important in patients who are HBeAg negative and this method is also applied to monitor treatment response to antiviral and to detect occult HBV infections immune control phase and also to detect reactivation of HBV cases.

Keywords: Hepatitis B Virus, HBsAg, HBeAg, Anti -HBe, RT -PCR

INTRODUCTION

Hepatitis B virus (HBV) infection is a significant public health concern and a leading cause of chronic liver disease, resulting in approximately 1.1 million deaths in 2022. The primary causes of these fatalities are cirrhosis and liver cancer. According to the 2022 report by the World Health Organization (WHO), it was estimated that 254 million individuals were living with chronic hepatitis B infections. Notably, there is a disproportionately high prevalence of HBV in low- and middle-income

countries, with 65% of the affected population residing in the African and Western Pacific regions. The control of HBV infection is a crucial objective for public health in regions where the virus is widespread.[1-5] Hepatitis B virus belongs to the Hepadnaviridae family and is a double-stranded DNA virus with a length of approximately 3,200 base pairs.[6-8] The cccDNA, or covalently closed circular DNA, can serve as a source of renewed virus production after the immune response to the acute infection has subsided. As a result, it forms a reservoir of infectious viral particles, contributing to the development of chronic hepatitis in specific individuals.[9,10] Detection of HBV-DNA can occur approximately 21 days prior to the typical appearance of HBsAg in the serum.[11] Hepatitis B is a multifaceted disease that requires a comprehensive assessment involving serological, biochemical, molecular, and histological evaluations for accurate diagnosis.[13] In recent studies, it has been demonstrated that the level of HBV-DNA in serum or plasma is closely associated with the biochemical and histological indicators of the disease, providing a more precise reflection of HBV replicative activity. As a result, the measurement of HBV-DNA in serum has emerged as a crucial tool for identifying individuals with high viral replication and for monitoring patients undergoing therapy.[3] Various quantitative assays are currently employed to measure the level of HBV-DNA load in serum samples, with real-time PCR being particularly recognized for its high sensitivity. The presence of HBV-DNA persists both during the acute and chronic phases of the disease. Its detection is more accurate than that of HBeAg, often appearing before the biochemical evidence of hepatitis.[13]

MATERIALS AND METHODS

The present cross sectional study was conducted in OPD of Gastroenterology and General Medicine, GSL Medical College and General Hospital, Rajahmundry, Andhra Pradesh in the period of March 2025 to June 2025. The study population consisted of individuals who tested positive for HBsAg, encompassing both genders with an age limit of up to 65 years. A total of 81 HBsAg positive patients were recruited in the study. Patient demographics, clinical history, and written consent were obtained from all study participants, in addition to receiving approval from the Institutional Ethical Committee. Nucleic acid extraction was done from each sample using Huwel Nucleic acid extraction kit (Huwel Life sciences Pvt Ltd, Hyderabad), qualitative and quantitative analyses were done by RT PCR, HBeAg and Anti-HBe were done by ELISA method in molecular and microbiology Laboratories, department of Microbiology, GSL Medical College and General Hospital, Rajahmundry.

HBV DNA detection and quantification using RT PCR: The detection was done using Huwel HBV quantitative qPCR Kit (Huwel Life Sciences, Hyderabad), linear range 1.4 X 106 to 7.2 X10-2 IU/ µL.

Nucleic acid extraction: Nucleic acid Extraction was done from 200µl serum using Nucleic acid extraction kit followed the kit manufactures instructions. Extracted elute was used for PCR.

Real Time PCR: The HBV Quantitative qPCR Kit is designed for immediate use in real-time PCR analysis. It includes a master mix with all necessary reagents and enzymes to enhance the efficiency of the RT-PCR process, as well as HBV gene-specific primers labeled with fluorescent markers. Furthermore, the Quantiplus HBV quantitation qPCR kit incorporates a secondary amplification system known as an internal control to detect and address potential PCR inhibition. The 15µL master mix contained buffer, dNTP's mix, primer probe and enzyme and 10µL elute was added to master mix. Correspondingly, 10 µl of 5 quantitation standards were used as positive controls and 10 µl of water as a negative control. The RT-PCR cycling parameters consisted of denaturation at 95º C for 1 minute followed by 45 cycles consisting of 95º C for 10 sec and 60º C for 10 seconds.. The problem of contamination was avoided by using pre-sterilized filtered micro tips and the reaction was run in a closed PCR work station. The amplification detection was carried out in a Quant Studio 5 RT PCR system. The RT-PCR uses target amplification techniques in which quantitation takes place during the exponential phase of amplification reaction.

RESULTS

A total of 81 HBsAg positive patients were recruited for the present study. Among them, HBV-DNA was detected among 54(66.6%) patients. Twenty seven (33.4%) patients were negative for the test (Table-I): Detection of HBV-DNA by RT PCR among HBsAg positive patients (n=81).

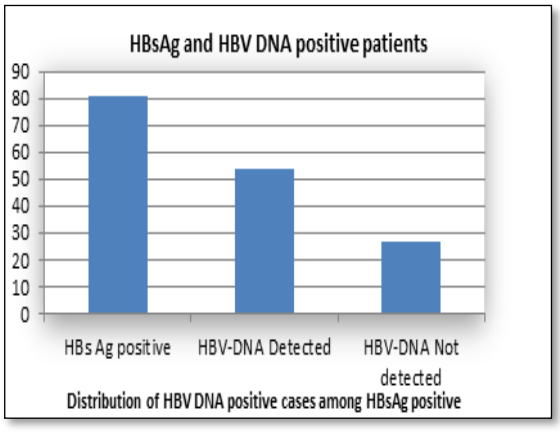

Figure 1- shows graphical representation of HBsAg vs HBV DNA

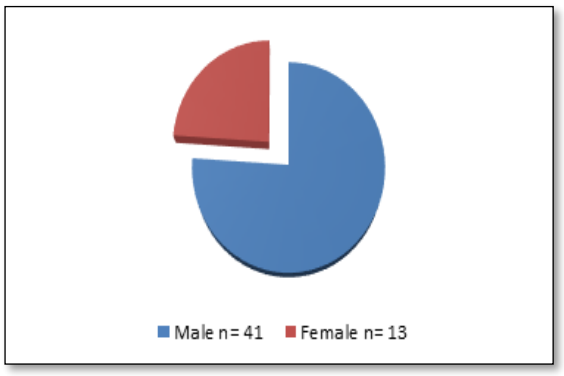

Figure 2- shows gender distribution in HBV DNA positive patients

Among 54 HBV-DNA positive patients 41 patients (76%) were male and 13 patients (24%) were Female (Table-II). Mean age of these 54 patients was 36±12 years.

Table 1: Detection of Hbv-Dna by Rt Pcr Among Hbsag Positive Patients (N=81).

Total patients	HBs Ag positive	HBV-DNA Detected	HBV-DNA Not detected
N = 81	81(100%)	54 (66.6%)	27 (33.4%)

Table – 1 shows HBV DNA vs HBe Ag

Table 2: Distribution of Sex in HBV DNA Positive Patients.

Sex	Total (n=54)	Percentage
Male	N= 41	76%
Female	N= 13	24%

Table – 2 shows HBV DNA Positive in patients

Table 3: Detection of HBV-DNA by RT-PCR and Detection of Hbeag, Anti-Hbe by Rapid in Hbsag Positive Patients (N=54).

Detection of HBVDNA	HBeAg positive	Anti-HBe positive	Both HBeAg & anti-HBe negative
Detected (54)	19 (35.2%)	24(44.4%)	11(20.4%)
Not Detected (27)	0 (0%)	14 (51.8%)	13(48.1%)
Total	19 (23.4%)	38(46.9%)	24(29.6%)

Table – 3 shows HBV DNA vs HBe Ag vs Anti HBe

Out of 81 HBsAg positive patients, HBV-DNA was detected in 54 patients. Among these, 19 (35.2%) patients were HBeAg positive, 24 (44.4%) patients were anti-HBe positive and 11 (20.4%) were negative for both HBeAg and anti-HBe. Age limit of patients was up to 65 years. HBV-DNA positive patients showed male predominance; 41 (76%) patients were male and 13 (24%) patients were female. Mean age of the patients was 36±12 years. Among 81 HBsAg positive patients, twenty four were receiving antiviral therapy. Out of them, HBV-DNA was decreased among 11 patients and could not be detected among 13 patients.

DISCUSSION

The detection of viral DNA in a patient's serum sample is a robust confirmation of the presence of infectious virion. HBV DNA levels can typically be detected within 30 days after exposure to the infection. These levels generally peak at the time of acute hepatitis and then gradually decrease, eventually disappearing when the infection resolves spontaneously. In the present study, out of 81 patients who tested positive for HBsAg, HBV DNA was detected by RT PCR in 54 patients (66.6%), while 27 patients (33.4%) tested negative for HBV DNA. This finding is consistent with a previous study by Rahman W et al., which was conducted among 56 HBsAg positive patients. In that study,

HBV-DNA was detected in the serum of 60.7% of patients, while 39.3% tested negative for HBV-DNA.[14] Additionally, a study by Ali Koyncver revealed similar results among HBsAg positive patients, with 58.3% testing positive for HBV-DNA in the serum and 41.7% testing negative for HBV-DNA.[15] The findings of this study also align with the studies of Danta et al and Yun-Fan et al.[14,16] Out of 54 HBV DNA positive patients, 35 (64.8%) tested negative for HBeAg, which is consistent with the findings of the study by Maimuna E Mendy. Maimuna E Mendy's study revealed that HBV-DNA was detected in 77% of HBeAg negative patients and all HBeAg positive patients.[17] Out of 27 (33.3%) HBV DNA negative patients, none tested positive for HBeAg, while 14 (51.8%) were found to be anti-HBe positive. This suggests that these

patients might have a silent infection, in line with the findings of ME Mendy et al.[17] Furthermore, 13 (48.1%) patients tested negative for both HBeAg and anti-HBe, indicating the possibility of precore mutant variety or carriers, which aligns with the study conducted by Yun-Fan et al.[16] In this particular study, among the 81 HBsAg positive patients, 19 (35.2%) tested positive for HBeAg, consistent with the findings of Chopra GS et al, where 26% of HBsAg positive patients were found to be HBeAg positive.[2] Recent data suggests that the presence of HBeAg and anti-HBe antibodies may not always accurately reflect the level of HBV-DNA in a patient's serum. Therefore, the assay may provide valuable information for HBeAg negative patients but still carry the HBV virion.[19] This is particularly important because precore point mutant HBVs can cause the HBeAg negative phenotype regardless of their replication status.[2,20] Furthermore, using the PCR method, it has been discovered that 70% to 85% of individuals with anti-HBe antibodies have detectable viral DNA in their circulation, indicating some level of ongoing viremia.[21] Out of 54 HBV DNA positive patients, 41 (76%) were male and 13 (24%) were female, showing a clear male predominance. This observation is consistent with a study by Rahman W et al.[14] The average age of these 54 patients was 36±12 years, aligning with similar findings in a study by Azita Ganji et al., which also demonstrated male predominance (72.0%) and an average age of 39±11 years.[22] The detection and quantification of HBV-DNA are crucial for diagnosing and monitoring HBV infection, as well as evaluating the response to antiviral therapy in patients with chronic Hepatitis B.[23] It's important to note that asymptomatic HBsAg positive carriers should not be assumed to have inactive disease; they should be regularly monitored every 3 to 6 months to assess disease activity and the development of complications. The majority of these carriers actually suffer from chronic hepatitis rather than acute hepatitis, likely contracting the disease during the perinatal period or early childhood.[18] HBV-DNA persists throughout the natural history of chronic hepatitis B, even in patients who show serologic evidence of viral clearance. It's worth noting that treatment generally does not lead to complete eradication of HBV from the body; continuous long-term therapy is necessary to maintain effective viral suppression and symptom control.[24] In this current study, out of the 24 patients receiving treatment, the viral load decreased in 11 patients, accounting for 45.8% of the total, while it was undetectable in 13 patients, constituting 54.2% of the total, after effective treatment. These findings align with a study conducted by Chopra GS et al., which reported a 63% decrease in viral load. The antiviral medications administered to these patients included nucleoside analogs like Entecavir, Lamivudine, and Telbivudine. These medications were utilized for varying durations, ranging from 3 months to 10 years, based on the individual requirements of the patients.

CONCLUSION

The prevalence rates of HBV vary across the world, with the highest rates being observed in eastern Asia. The quantitative HBV RT-PCR assay has high sensitivity, specificity, accuracy, wide linearity, and good reproducibility, combined with a small sample volume requirement. These characteristics make it well suited for application to large clinical and epidemiological studies in routine diagnostic laboratories.

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