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Validation of quantitative hepatitis B virus DNA and hepatitis B e antigen titers in patients of chronic hepatitis B virus infection on entecavir therapy at tertiary care hospital

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ABSTRACT

Background: The management of chronic hepatitis B (CHB) infection is complex due to its natural history, which includes fluctuating viral replication and associated hepatic dysfunction. **Aims and Objectives:** The study was conducted to evaluate quantitative hepatitis B virus (HBV) DNA and hepatitis B e antigen (HBeAg) titers in patients of chronic HBV infection on entecavir and to find the rate of hepatitis B surface antigen (HBsAg)-positive patients among clinically suspected cases of hepatitis. The objective of the study was to find the rate of HBeAg-positive patients in HBsAg-positive cases on entecavir and to correlate the level of alanine aminotransferase (ALT) with status of HBeAg and HBV DNA level in HBsAg-positive cases on entecavir. **Materials and Methods:** The study population consisted of 87,600 patients from MG hospital, affiliated with RVRS Medical college in Bhilwara, Rajasthan. Screening of HBsAg was performed using rapid card test method, HBeAg was done by enzyme-linked immunosorbent assay, and HBV DNA was quantified by real-time polymerase chain reaction. Aspartate aminotransferase and ALT were also measured. **Results:** Blood samples from 87,600 individuals who had a clinical suspicion of having hepatitis B were used in the study. A total of 3650 people (4.1%) had positive HBsAg tests. Of the 3650 (4.1%), 312 (8.54%) experienced a chronic illness. 109 (312) showed positive HBeAg, 102 (312) had elevated ALT enzyme levels, and 117 (312) had positive HBV DNA among the 312 people with CHB. **Conclusion:** In conclusion, the findings of our study indicate a low prevalence of CHB virus infection.

Key words: Hepatitis B surface antigen; Chronic hepatitis; Hepatitis B virus DNA; Entecavir; Hepatitis B e antigen

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INTRODUCTION

Chronic hepatitis B (CHB) remains a substantial public health problem and the leading cause of hepatocellular carcinoma (HCC) worldwide.[1,2] According to the World Health Organization (WHO) statistics, approximately 0.257 billion people are infected with the hepatitis B virus (HBV) worldwide, with around 0.88 million deaths annually, which causes a huge medical and economic burden.[3] The WHO estimates that 254 million individuals worldwide have CHB in 2022.[4] "The WHO has set a plan to eradicate viral hepatitis as a public health threat by 2030, aiming for a

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90% reduction in new CHB infections and a 65% reduction in CHB-related mortality."[5]

Monitoring the progression of hepatitis B infection becomes essential since individuals with persistent HBV infection have an increased risk of consequences such fibrosis, liver cirrhosis, and occasionally cancer.[6]

Hepatitis B surface antigen (HBsAg) is used as a marker of infection and its levels fluctuate throughout the different stages of illness. It can also be used to track how well a CHB virus infection is responding to therapy.[7] It is commonly known that the presence of hepatitis B e antigen (HBeAg) may be a sign of active viral replication. Measurements of viral replication and infection status have become more precise with the development of molecular testing and the increased accessibility of quantitative HBV DNA levels.[7]

The purpose of the study is to evaluate quantitative HBV DNA and HBeAg titers in patients of chronic HBV infection on entecavir from a rural tertiary care hospital, to find the prevalence of HBsAg-positive patients among clinically suspected cases of hepatitis, to find the rate of HBeAg-positive patients in HBsAg-positive cases on entecavir, to correlate the level of alanine transaminase (ALT) with status of HBeAg and HBV DNA level in HBsAg-positive cases on entecavir, and to evaluate the efficacy of HBeAg titer and HBV DNA titer in HBsAg-positive patients on entecavir as a treatment predictor and to see some goals of treatment for chronic HBV which include: Reducing inflammation in the liver, suppressing the virus, improving serologic and histologic markers, preventing complications such as cirrhosis and liver cancer, and preventing transmission of the virus to others.

Aims and objectives

The study was conducted to evaluate quantitative HBV DNA and HBeAg titres in patients of chronic HBV infection on entecavir. The objective of the study was to find out the rate of HBeAg positive patients in HBsAg positive cases on entecavir and to correlate the level of ALT with status of HBeAg and HBV DNA level in HBsAg positive cases on entecavir.

MATERIALS AND METHODS

Study design

A prospective cross-sectional study design was used for validating the quantitative HBV DNA and HBeAg titers in patients of chronic HBV infection on Entecavir.

Study setting

The present study was primarily conducted in the Department of Microbiology, Rajmata Vijaya Raje Scindia Medical College (RVRS) Medical College Bhilwara, Rajasthan.

Duration of study

18 months.

Inclusion criteria

All HBeAg-positive samples from both male and female patients irrespective of age from patients of chronic HBV infection on entecavir.

Exclusion criteria

Patients with other hepatotropic organisms or any liver injury by any means such as drugs and alcohol

Ethical clearance

The study was conducted after obtaining the ethical clearance from the institutional ethics committee of Malwanchal University, bearing protocol number (MU/Research/EC/Ph.d./2020/27).

Data collection

History and clinical details of the patients were collected.

Specimen

Blood Sample: A whole blood sample from a patient suspected of having an HBV infection was taken on an ethylenediaminetetraacetic acid (EDTA) tube in a BD container. The sample was properly labeled and delivered to the Microbiology Central Laboratory at the MG Hospital affiliated with RVRS Medical College RVRS, Bhilwara, Rajasthan, along with the completed requisition papers.

Specimen preservation

The appropriately labeled screw-capped tubes were closed tightly and frozen at −80°C before analysis of viral load by quantitative polymerase chain reaction (PCR).

Specimen processing

Whole blood sample in an EDTA container was centrifuged at 2000 rpm for 10 min at +4□ in a refrigerated centrifuge. The separated plasma was aliquoted directly into appropriately labeled sterile storage vials in multiple aliquots.

Processing of blood specimens for HBsAg was done by rapid card test (J. Mitra and Co. Pvt. Ltd., Hepacard kit), For HBeAg enzyme-linked immunosorbent assay (DIA. PRO) was done and HBV DNA was detected in all samples using PCR (QIAamp DNA Mini kit).

Statistical calculations

As data were coded, entered, and then analyzed using the Statistical Package for the Social Sciences (SPSS) version 20.0. Categorized data were expressed as frequency and percentages. Chi-square test was applied to find association between variables. A $P<0.05$ was considered statistically significant.

RESULTS

Blood samples from clinically suspected hepatitis B patients (n=87,600) were included in the study. Of the total sample, 3650 (4.1%) were found to be HBsAg positive. Among these HBsAg-positive patients, 312 (8.54%) developed CHB.

Among CHB patients, 117 (37.5) tested positive for HBV DNA, 102 (32.69) patients had elevated level of ALT enzyme, and 109 (34.9) were HBeAg positive.

Figure 1 shows the group at high risk for a chronic hepatitis infection. Overall, 12/312 (3.84%) were voluntary blood donors, 1/312 (0.32%) were commercial sex workers, 22/312 (7.05%) were technicians, 18/312 (5.7%) were doctors, and 65/312 (20.8%) were paramedical personnel.

Figure 2 illustrates the clinical circumstances of patients with chronic hepatitis. The most frequent symptoms were jaundice (66.3%), nausea (66.6%), and vomiting (66.6%). 66.3% appetite loss, 30.4% weight loss, 18.5% edema, 10.2% ascites, 8.97% spider angiomas, and 3.8% coagulopathy.

Out of total 312 chronic hepatitis patients, 162 (100%) patients had persistently elevated liver enzymes. Among them, 120 (74.1 %) had viral load above 20 lakhs and 42 (25.9%) had viral load below 20 lakhs. The observed difference between viral load and liver enzymes was found to be statistically significant (P=0.00001).

Table 1 shows that out of 312 chronic hepatitis patients, 237 (76%) patients were on entecavir therapy and 75 (24%) patients lost to follow-up.

Table 2 shows that out of 312 chronic hepatitis patients, 32 (10.2%) were having liver cirrhosis and 4 (1.2%) were having hepatocellular carcinoma.

Table 3 illustrates that out of 237 patients on entecavir, at 48 weeks, a total of 148 (62.4%) achieved undetectable HBV DNA, 152 (64.1%) showed normal level of ALT, and out of 109 HBeAg-positive patients, 21 (19.2%) showed HBeAg seroconversion and at 96 weeks, 189 (79.7%) achieved undetectable HBV DNA, 198 (83.5%) showed normal level of ALT, and out of 109 HBeAg-positive patients, 34 (31.1%) showed HBeAg seroconversion.

DISCUSSION

This research implies that anti-HBV medication may effectively stop the advancement of chronic liver disorders by providing long-term reduction of HBV replication. Therefore, the most crucial therapeutic objective for CHB patients should be the inhibition of HBV replication.

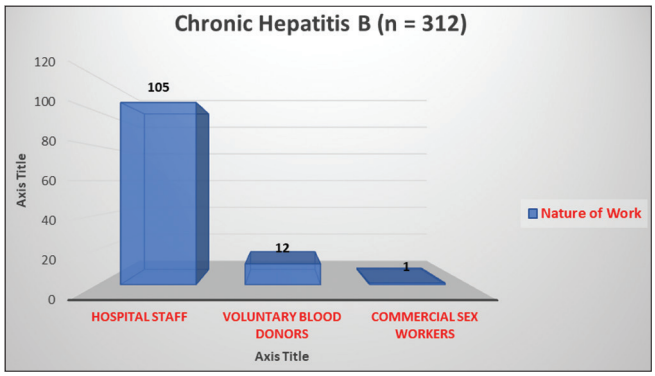

Figure 1: Assessment of high-risk group in chronic hepatitis infection

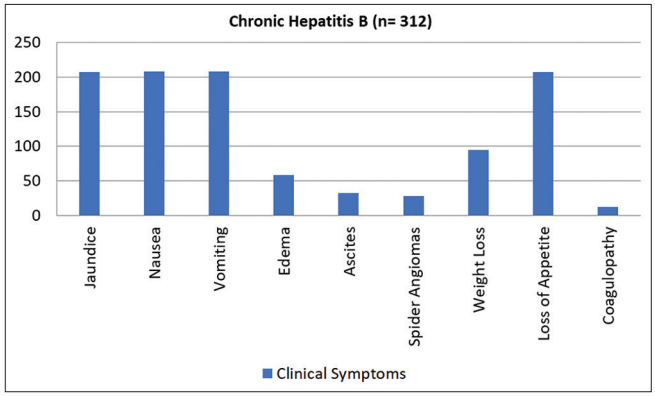

Figure 2: Clinical symptoms among chronic hepatitis patients

Table 1: Distribution of clinically suspected chronic hepatitis patients in relation to treatment with entecavir (n=312)

Total HBsAg positive after 6 months	Patient on entecavir therapy	Patient lost to follow-up
312	237 (76%)	75 (24%)

HBsAg: Hepatitis B surface antigen

Table 2: Clinical complications in chronic hepatitis patients (n=312)

Clinical conditions	Chronic hepatitis n (%)
Liver cirrhosis	32 (10.2)
Hepatocellular carcinoma	4 (1.2)

In the present study, analysis of HBV DNA load showed that 117/312 (37.5%) were positive for Viral DNA, 109/312 (34.9%) were positive for HBeAg, and 102/312 (32.6%) have elevated ALT (Table 4). This was comparable with the study by Chopra et al., wherein 33% of the samples were positive for HBV-DNA and (24/91) 26% of samples were positive for HBeAg.[8] According to research by Amarapurkar et al., 39% of HBeAg-positive patients were segregated from HBsAg-positive patients.[9] Lee et al., have, however, also reported a greater isolation rate of HBeAg (60.6%).[10] HBV DNA positivity was highest in Hasan et al., (44.8%) and Rabbi et al., (40.2%),

Table 3: Efficacy outcomes endpoint at weeks 48 and 96 in chronic hepatitis patients on entecavir therapy

Efficacy endpoint	n	At week 48 n (%)	At week 96 n (%)
Undetectable HBV DNA	237	148 (62.4)	189 (79.7)
ALT normalization	237	152 (64.1)	198 (83.5)
HBeAg loss and seroconversion	109	21 (19.2)	34 (31.1)

HBV: Hepatitis B virus, ALT: Alanine aminotransferase, HBeAg: Hepatitis B e antigen

Table 4: Occurrence of HBV DNA, ALT, and HBeAg in chronic hepatitis patients (n=312)

S. No.	Parameters	Chronic hepatitis patients n (%)
1	HBV DNA	117 (37.5)
2	ALT	102 (32.69)
3	HBeAg	109 (34.9%

HBV: Hepatitis B virus, ALT: Alanine transaminase, HBeAg: Hepatitis B e antigen

respectively.[11,12] An early phase of chronic HBV infection can occasionally be identified by strong HBV replication, low aminotransferase levels, and modest histologic activity, as well as an HBeAg-positive result. However, HBeAg seroconversion and the development of the corresponding antibody (anti-HBe) may occur during the course of a chronic HBV infection. This event is typically associated with the infection's transition from a high-replication phase to an inactive phase, during which there is little to no residual viral replication and essentially normal liver histology. Not all patients who lose HBeAg and seroconvert to anti-HBe antibody, however, experience a long-lasting reduction in the activity of liver disease and HBV replication.[13]

Of the patients with chronic hepatitis in the current study, 65/312 (20.8%) were paramedics, and 22/312 (7.05%) were technicians (Figure 1). This was similar to Puri's, study, which also revealed that HCWs had the highest overall HBsAg-positive rates (2.4%), indicating more exposure to blood and other known risk factors.[14]

As opposed to our research, Sharma et al., 85 (12.7%) findings indicated that high-risk groups were linked to HBsAg positivity and unprotected sex as well as STDs.[15] The cause could be that hepatitis B is not genetically or inherited; instead, it can only be contracted by direct blood contact or sexual contact. This means that several family members may be infected without realizing it.

Furthermore, the clinical symptoms associated with chronic hepatitis patients were also tabulated in the study. Jaundice 207/312 (66.3%), nausea 208/312 (66.6%) followed by vomiting 208/312 (66.6%) were the most common (Figure 2). The study conducted by Wani et al., showed that majority (78.05%) of the patients had asymptomatic transaminitis, and only 21.9% patients had symptoms such as fatigue, jaundice, nausea, vomiting, and anorexia.[16]

In the current study, 162 patients had persistently elevated liver enzymes. Among them, 74.1% had viral load above 20 lakhs and 25.9% had viral load below 20 lakhs. The observed difference between viral load and liver enzymes was found to be statistically significant (P=0.00001) (Table 5). The result was in accordance with the study conducted by Esmaeelzadeh et al., who also showed that HBV-DNA levels >10^5 IU/mL were associated with higher ALT and AST serum levels.[17] A study by Tufon et al., also showed that there was a significantly higher proportion of patients with elevated liver aminotransferases (ALT and AST) having viral loads >20,000 IU/mL.[18]

The gift looks at suggests that out of 312 persistent hepatitis sufferers, 32 (10.2%) had been having liver cirrhosis and 4 (1.2%) had been having HCC (Table 2). This turned into similar to the look at through Saravanam et al., who confirmed 59% (37/63) with liver cirrhosis and 6 instances with HCC, 33% (2/6).[19] A look at through Tsukuma et al., additionally confirmed 12.5% instances of liver cancer.[20] A latest all-India survey discovered that 43% of HCC instances are HBV associated;[21] occurrence among HCC instances is 42% within side the south;,[22] 39–69% within side the north;[23] and 82% within side the west.[24]

Table 3 illustrates that out of 237 affected people on entecavir, at 48 weeks, a complete of 148 (62.4%) accomplished undetectable HBV DNA, 152 (64.1%) showed normal level of ALT, and out of 109 HBeAg sufferers, 21 (19.2%) confirmed HBeAg seroconversion and at 96 weeks, 189 (79.7%) accomplished undetectable HBV DNA, 198 (83.5%) showed normal level of ALT and out of 109 HBeAg sufferers, 34 (31.1%) confirmed HBeAg seroconversion. Our consequences are akin to the enjoy of others. A study by Saikia et al., additionally confirmed undetectable serum HBV DNA with a PCR assay (67% vs. 36%, P<0.001) and normalization of ALT (68% vs. 60%, P=0.02). The suggest discount in serum HBV DNA from baseline to week 48 became more with entecavir.[25] A examine with the aid of using Ashqar et al., confirmed usual reaction to ETV at 48 weeks with an undetectable HBV DNA became 46.5%. When the remedy became prolonged past

Table 5: Association of viral load and liver enzymes in chronic hepatitis patients (n=312)

Viral load	Normal/decreased n (%)	Persistently elevated n (%)	Total n (%)	P-value
Above 20 lakhs	55 (35.4)	120 (74.1)	175 (56)	<0.00001
Below 20 lakhs	95 (63.3)	42 (25.9)	137 (44)	
Total	150 (100)	162 (100)	312 (100)	

48 weeks, with an average of 24 months, the general reaction became 67.4% and a pair of out of 15 (13.3%) HBeAg-fantastic sufferers have become HBeAg bad after 4 years of remedy.[26] A retrospective multicenter examine, concerning 25 Spanish centers, dealt with a hundred ninety remedy-naive persistent hepatitis B sufferers. In that examine, undetectable HBV DNA at 48 weeks became 83% (61% HBeAg-fantastic; 92% HBeAg-bad). Retrospective multicenter study from 5 Argentine centers in 69 previously untreated chronic HBV patients treated with ETV for a mean of 110 weeks. The rate of undetectable HBV DNA was 77% in that group at week 48. In a cohort of 154 patients treated with ETV at King's College London, 76% had HBV DNA and 12 IU/mL.[26]

A study by Gish et al., also showed the efficacy of entecavir, when continued for up to 96 weeks. Within the 2nd-year treatment cohort (virologic responders at week 48), The proportion of patients achieving an undetectable HBV DNA level increased from 64% at week 48% to 74% at the end of dosing. This 10% increment in virologic response was accompanied by a 13% incremental increase in normalization of ALT levels (79% at the end of dosing) and by an additional 11% of patients experiencing HBeAg seroconversion. The findings in the 2nd-year cohort confirmed that extended treatment with entecavir provided continued viral suppression and normalization of ALT levels through 96 weeks.[27] A study by Nayak et al., showed that the mean HBV DNA at baseline was 5.99 which decreased to 2.12 log IU/mL at the end of 40 weeks. Thus, there was a mean change of 3.87 log IU/mL which was statistically significant (P<0.0001). Out of 140 HBeAg-positive subjects, 50 (35.71%) had become negative at the end of 40 weeks. Only 10.71% of the patients had normal ALT (<40 IU/L) values in the beginning which increased to 100% at the end of 40 weeks.[28]

Limitations of the study

HBV biomarkers, such as hepatitis core antigen (anti-HBc), hepatitis B surface antibody (anti-HB), and hepatitis B e antibody (anti-HBe), have been used for the detection of infective phases of HBV but due to lack of resources we were unable to do these tests. Others factors that may affect the accuracy of HBsAg tests include genotypes and co-infection with hepatitis C or HIV. Most importantly, the major drawback of the present study is that, as this is a cross-sectional study, and no longitudinal data is available, therefore, major conclusions cannot be drawn regarding casuality.

CONCLUSION

In conclusion, the findings of our study indicate a low prevalence of CHB virus infection. Paramedical workers (20.8%) were associated with high-risk group in chronic hepatitis in our study. Entecavir significantly improves virological, biochemical, and serological markers in HBeAg-positive treatment-naïve CHB patients.

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Authors' Contributions:
DB- Definition of intellectual content, literature survey, prepared first draft of manuscript, implementation of study protocol, data collection, data analysis, manuscript preparation and submission of article; **VAS-** Concept, design, clinical protocol, manuscript preparation, editing, and manuscript revision; **MR-** Review manuscript; **VS-**Literature survey and preparation of figures; **PS-** statistical analysis.

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