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Assessing the Relationship between HBV DNA, HBeAg and ALT Levels in Patients with Chronic Hepatitis B

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ABSTRACT

Chronic Hepatitis B (CHB) caused by the Hepatitis B virus (HBV) is a major global health concern with a substantial disease burden. CHB contributes to over 240 million chronic infections and approximately 600,000 deaths annually. Understanding the factors associated with CHB progression is crucial for effective management. This study aimed to investigate the correlation between HBV DNA levels, Hepatitis B e-Antigen (HBeAg) status and Alanine Aminotransferase (ALT) levels in 200 clinically and serologically confirmed CHB patients. A cross-sectional study was conducted at the Departments of Microbiology and Gastroenterology, Guntur Medical College/Government General Hospital, Guntur. The study enrolled 200 CHB patients whose diagnoses were both clinical and serological. Serum ALT levels were quantified using an automated Beckman Coulter system. HBeAg was detected through Chemiluminescence Microparticle Immunoassay (CMIA) and HBV DNA levels were measured using real-time PCR with the Alto Star HBV PCR Kit. Among the CHB patients, 68% were male, with the predominant age group being 31-40 years (32%). HBV DNA was detected in 14.5% of cases, 21% tested positive for HbeAg and 28% had abnormal ALT levels. Notably, 21% of patients were positive for both HBeAg and HBV DNA and they exhibited abnormal ALT levels. However, 8% of patients had abnormal ALT levels despite being HBeAg negative and having undetectable HBV DNA. This study underscores a significant association between HBV DNA levels, HBeAg status and ALT elevations in CHB patients. Elevated HBV DNA levels were more frequent in HBeAg-positive patients, who consistently displayed raised ALT levels. Concurrent monitoring of these markers is essential for effective CHB management, facilitating timely intervention and better patient outcomes in this global health concern. Chronic hepatitis B, HBV DNA, HBeAg, alanine aminotransferase, ALT.

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Key Words
Chronic hepatitis B, HBV DNA, HBeAg, alanine aminotransferase, ALT

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INTRODUCTION

Hepatitis B virus (HBV) infection encompasses a spectrum of liver diseases, including acute and chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC)[1]. Accurate diagnosis and confirmation of HBV infection heavily rely on the detection of serological markers, with hepatitis B surface antigen (HBsAg) in serum being widely recognized as the most reliable indicator of HBV infection.

A hallmark of chronic hepatitis B (CHB) infection is the persistent presence of HBsAg seropositivity, lasting for at least six months. In addition to HBsAg, quantifying HBV-DNA levels serves as another valuable marker for assessing CHB infection[2,3]. HBV-DNA measurement also plays a critical role in monitoring the virus's suppression in patients undergoing treatment[4,5].

The natural course of CHB involves distinct phases, starting with an HBeAg-positive, immune-tolerant phase, progressing to an HBeAg-positive, immune-reactive phase, followed by an HBeAg-negative, inactive HBV carrier state, HBeAg-negative CHB phase, and ultimately an HBsAg-negative phase (referred to as occult infection)[6]. HBV as a DNA virus, possesses the capacity for genome organization a replication cycle and an impact on the host immune response. Detection of HBV can occur serologically, through virus isolation, identification of HBV DNA polymerase activity and detecting HBV DNA in the serum[7].

Histopathological changes observed in HBV infection include neuroinflammatory activity (characterized by inflammation and necrosis) and fibrosis, which are closely associated with HBeAg, anti-HBe, ALT and HBV DNA levels[8-9]. Clinical guidelines recommend using cutoff values of 40 IU mL^{-1} for alanine aminotransferase (ALT) levels and 2000 IU mL^{-1} for HBV DNA levels[10,11]. A histologic activity index has been established, utilizing histological features to assess both the grade (inflammation) and stage (fibrosis) of liver disease.

The objective of our study is to investigate the correlation between HBV DNA load and ALT levels in HBeAg-positive patients and evaluate the utility of serum ALT and HBV DNA levels as screening tools for liver disease.

MATERIALS AND METHODS

Location and study period: This research was conducted at the Government General Hospital in Guntur, Andhra Pradesh, India, during the study period spanning from March 2021-2022.

Sample collection: Aseptic measures were strictly followed during the collection of samples. A total of 10 mL of blood was aseptically drawn from each patient through venipuncture, using a disposable syringe. The collected blood was then transferred into sterile vacutainers without anticoagulants. Subsequently, these vacutainers were allowed to stand undisturbed at room temperature for a period ranging from 45 min 1 hrs. Following this, serum was extracted by centrifuging the blood samples at 500x g for 15 min. The resulting serum samples were divided into 2 mL aliquots and stored at -80°C in a refrigerator for further analysis.

Liver function tests: Serum samples were subjected to liver profile tests using a Beckman Coulter AU 480 autoanalyzer. Routine investigations, such as Bilirubin, Total Protein, Albumin, Globulin, Alanine Aminotransferase, Aspartate Aminotransferase and Alkaline Phosphatase, were analysed following standardized procedures.

Confirmation of HBsAg: The presence of the hepatitis B surface antigen (HBsAg) was confirmed using the HEPALISA Kit, which employs an enzyme-linked immunosorbent assay (ELISA). This assay is highly sensitive and specific, allowing for the detection of HBV infection.

Detection of HBeAg (CMIA): Chronic hepatitis B (CHB) patients were screened for the hepatitis B e antigen (HBeAg) using the Chemiluminescent Microparticle Immunoassay (CMIA) method with the Architect CMIA kit. HBeAg is an important marker that provides insights into the replicative activity of the hepatitis B virus in the patient's body.

DNA quantification: Quantification of HBV DNA was carried out using the Alto Star HBV PCR kit 1.5. This kit employs a polymerase chain reaction (PCR) approach to measure the amount of HBV DNA in the patient's serum. It is a precise method for assessing viral load, which is crucial for monitoring the progression of hepatitis B and evaluating treatment efficacy.

Ethical considerations: The study adhered to rigorous ethical standards and obtained approval from the Institutional Ethics Committee (IEC) at Guntur Medical College and Government General Hospital in Guntur, Andhra Pradesh, India, with reference number GMC/IEC/176/2019. Ethical approval ensures that the study followed ethical guidelines, safeguarded patient rights and maintained confidentiality and informed consent procedures as required for human research.

Statistical analysis: The statistical analysis of the data involved descriptive statistics, including gender distribution, abnormal liver enzyme prevalence, age group distribution, HBeAg status and HBV DNA viral load.

RESULTS

In a cohort of 200 patients with chronic Hepatitis B (CHB) infection, gender distribution revealed that 68% (132) were males, while 32% (68) were females. Among these patients, various abnormalities in liver enzymes were observed, including.

Elevated Bilirubin levels were detected in 58.5% (117) of cases. Elevated Aspartate Aminotransferase (AST) levels were noted in 34.5% (69) of cases. Elevated Alanine Aminotransferase (ALT) levels were observed in 28% (56) of cases. Alkaline Phosphatase (ALP) levels were found to be elevated in 40% (80) of cases.

Furthermore, among the 200 patients the presence of Hepatitis B e Antigen (HBeAg) was identified in 21% (42) of cases and HBV DNA was detected in 14.5% of cases. These findings provide valuable insights into the gender distribution and liver enzyme abnormalities among CHB patients as well as the prevalence of HBeAg positivity in this cohort. Table 1 shows the Gender-Wise distribution of Chronic Hepatitis B virus cases. Among the 200 Chronic Hepatitis B cases enrolled in the study, 68% (132) were males and 32% (68) were females. The distribution of chronic hepatitis B cases by age group is as follows.

In the age group 11-20, there were two cases, which comprised 100% of this age group. No female patients in this age group were identified. This age group represented 1% of the total cases. For the age group 21-30, there were a total of 45 cases, with 28 cases among male patients and 17 cases among female patients. This age group accounted for 22.5% of the total cases. In the age group 31-40, there were 72 cases in total, including 48 male patients and 24 female patients. This age group constituted 36% of the total cases. The age group 41-50 had 53 cases in total, with 35 cases among male patients and 18 cases among female patients. This age group represented 27% of the total cases. For the age group 51-60, there were 22 cases in total, with 15 cases among male patients and 7 cases among female patients. This age group accounted for 11% of the total cases. In the age group 61-70, there were 5 cases in total, including 4 male patients and 1 female patient. This age group represented 2.5% of the total cases. No cases were recorded in the age group 71-80 (Table 2 and 3).

Table 1: Gender-wise distribution of chronic hepatitis b cases

Total	Male	Female
N = 200	132 (68%)	68 (32%)

Table 2: Abnormal liver enzymes in chronic hepatitis B cases

Liver enzymes	Normal	Abnormal	Total
Bilirubin	83(41.5%)	117(58.5)	200(100%)
ALT	144(72%)	56(28%)	200(100%)
AST	131(65.5%)	69(34.5%)	200(100%)
ALP	120(60%)	80(40%)	200(100%)

Table 3: Age-wise distribution of chronic hepatitis B cases

Age group	Male (132) (68%)	Female (68) (32%)	Total 200 (100%)
11-20	2(100%)	0(0%)	2(1%)
21-30	28(62.2%)	17(37.8)	45(22.5%)
31-40	48(66.7%)	24(33.3%)	72(36%)
41-50	35(66%)	18(34%)	53(26.5%)
51-60	15(68.2)	7(31.8)	22(11%)
61-70	4(80%)	1(20%)	5(2.5%)
71-80	0(0%)	0(0%)	0(0%)

Table 4: Distribution of HBeAg positive case

Total	HBeAg positive	HBeAg negative
200	42(21%)	158 (79%)

Table 5: Distribution of HBV DNA positive cases viral load

Viral load range	No of samples
10-10	13(44.8%)
10-10	4(13.79%)
10-10	3(10.34%)
10-10	5(17.24%)
10-10	4(13.79%)
Total	29(100%)

HBeAg positivity: Among the 200 CHB patients, 42 (21%) were found to be HBeAg positive. This indicates that these patients have active viral replication and are potentially in an infectious phase of their disease.

HBeAg negativity: The majority of the CHB patients, 158 (79%) tested negative for HBeAg. This suggests that these patients may be in a different phase of the disease, such as the immune-reactive or inactive carrier phase, where viral replication is reduced or controlled by the immune system (Table 4).

Quantification of HBV DNA by RT PCR: DNA was quantified by using Alto Star HBV PCR kit 1.5. Viral Load Range 10-10. There were 13 patients (44.8% of the total) with a viral load in the range of 10,000-100,000 copies of HBV DNA per milliliter (mL) of blood.

Viral load range 10-10: Four patients (13.79% of the total) had a viral load ranging from 100,000-1,000,000 copies of HBV DNA per mL.

Viral load range 10-10 : Three patients (10.34% of the total) exhibited a viral load between 1,000,000 and 10,000,000 copies of HBV DNA per mL.

Viral load range 10-10: Five patients (17.24% of the total) had a viral load ranging from 10,000,000-100,000,000 copies of HBV DNA per mL.

Viral load range 10-10: Four patients (13.79% of the total) showed a viral load between 100,000,000 and 1,000,000,000 copies of HBV DNA per mL (Table 5).

Overall the study assessed the viral load in 29 CHB patients. The results indicate a diverse range of viral loads among these patients, with the majority falling into the lower viral load categories (10 -10 and 10-10). Monitoring viral load is crucial for understanding

disease progression and guiding treatment decisions in CHB patients, as higher viral loads are often associated with more active disease and a greater risk of complications.

DISCUSSION

The findings of this study provide significant insights into the demographic and clinical characteristics of patients with chronic Hepatitis B (CHB) infection. Understanding these characteristics is crucial for improving patient care and management strategies.

Gender distribution and implications: Our study observed a significant male predominance (66%) among patients with chronic Hepatitis B (CHB) (Islam SMRU *et al.*[12]. This finding aligns with previous research and raises intriguing questions about potential gender-related differences in healthcare-seeking behaviour or exposure to risk factors. One possible interpretation is that males may be less likely to seek medical attention for CHB-related issues or could have higher exposure to risk factors, such as certain occupations or lifestyle factors, that predispose them to CHB. Understanding these gender disparities is crucial as it may lead to the development of more gender-specific healthcare strategies. Tailoring interventions and educational campaigns to address these differences could potentially improve outcomes for both males and females with CHB.

Liver enzyme abnormalities: A critical finding in our study is the high prevalence of abnormal liver enzymes, including bilirubin, AST, ALT and ALP, in our cohort. These enzymes are reliable biomarkers of liver inflammation and damage. The presence of elevated liver enzymes underscores the need for regular and systematic monitoring of liver function in CHB patients. This finding aligns with Nita *et al*.[14], Ormeci *et al.*[15], Wong *et al*.[16], Günal *et al.*[17] and Sarin *et al.*[18]. Studies highlighting the importance of liver enzyme abnormalities as indicators of disease severity and progression in CHB. Regular liver function testing should be integrated into the standard management plan for CHB patients (Cholongitas and Others)[19]. By doing so, healthcare providers can detect liver damage early, enabling timely intervention and potentially improving patient outcomes (Bennett *et al.*)[20]. Monitoring liver enzymes is a fundamental aspect of CHB management[21].

Age distribution and disease prevalence: Our study revealed that the prevalence of CHB was highest in the age group of 31-40 years, followed by 41-50 years. This age-related pattern of CHB prevalence mirrors the global epidemiological pattern of the disease, where it predominantly affects individuals in their most productive years (Chen *et al.*)[22]. This observation has important implications for public health planning. It suggests that interventions and awareness campaigns should be targeted towards younger age groups who are most at risk of CHB[23]. However the lower prevalence in older age groups might indicate effective disease management in these cohorts, possibly due to earlier diagnosis and treatment. This age-related variation underscores the need for public health efforts to focus on the most affected age groups while also ensuring that older CHB patients receive appropriate care and support.

HBeAg status and Its clinical significance: Our study identified the presence of Hepatitis B e Antigen (HBeAg) in 21% of patients (Li *et al*.)[24] indicating active viral replication. In contrast the majority of patients were HBeAg-negative, suggesting different disease phases, such as the immune-reactive or inactive carrier phase (Chen et al.[22]). This variation in HBeAg status has significant clinical implications. Monitoring HBeAg is crucial because it provides essential information for tailoring treatment strategies. Studies have consistently linked HBeAg status to disease prognosis and treatment response (Karra *et al*.[25]). Tailored treatment plans based on HBeAg status can optimize therapeutic outcomes for CHB patients. Therefore, regular assessment of HBeAg status should be part of the standard clinical evaluation for individuals with CHB.

HBV DNA viral load diversity: Our study's finding of a diverse range of HBV DNA viral loads among CHB patients highlights the complexity of CHB management (Gupta *et al*.[26]). The majority of patients had lower viral loads, indicating varying degrees of viral replication. This distribution calls for personalized treatment approaches because higher viral loads are associated with more active disease and an increased risk of complications (Shao *et al*.[27]). It is imperative to recognize that CHB is not a one-size-fits-all condition, and treatment plans should be tailored to individual patient's viral load profiles. Regular monitoring of viral load is therefore essential, as it guides therapeutic decisions and may improve patient outcomes. A nuanced approach to managing CHB based on viral load is crucial for achieving the best clinical results.

Limitations: Our study's small sample size (100 patients) may not fully represent the broader CHB population, potentially introducing selection bias. We did not explore genetic or environmental factors contributing to disease progression. Further research with larger, diverse cohorts is needed. Our cross-

sectional design captures data at one point, limiting the ability to establish causality or track changes over time. Additionally, our study was conducted in a single center, potentially affecting generalizability to other settings.

Conclusion: This study illuminates the demographic and clinical profiles of chronic Hepatitis B patients, highlighting the necessity for healthcare strategies tailored to gender differences, consistent monitoring of liver functions and customized treatment plans considering age, HBeAg status and viral load levels. The findings underscore the critical need for further research to delve into the underlying causes of these observations. Such investigations are pivotal for enhancing CHB management, aiming to diminish the disease's overall burden and improve patient outcomes.

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