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Quantitative Analysis and Correlation of HBeAg, HBeAb, and HBV DNA in Chronic Hepatitis B Patients in Swat, Khyber Pakhtunkhwa

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Abstract

Aims: The research aims to determine quantitative values of HBeAg and HBeAb concentrations in chronic hepatitis B patients while assessing their relationship with serum hepatitis B DNA levels.

Place and Duration of Study: This study was conducted at Saidu Teaching Hospital, Swat. This study lasts for 6 months, from December 2023 to April 2024.

Methodology: This study comprised 100 patients with confirmed HBV DNA positive and a chronic hepatitis B. Exclusion criteria included those using antiviral medication, those with autoimmune or metabolic liver disorders, and those with co-infections (HCV, HIV, or HDV). HBeAg and HBeAb levels were assessed using CLIA, and HBV DNA was processed using qRT-PCR. Accuracy was ensured using internal quality control measures. To evaluate the correlations between HBV DNA, HBeAg, and HBeAb, data analysis was done using SPSS Version 17.0 and Pearson correlation. P-values less than 0.05 were regarded as statistically significant.

Results: The mean viral load of the 100 patients was 60,800 IU/mL, and all of them tested positive for HBV DNA. HBeAb was 8.75 IU/mL on average, while HBeAg was 12.82 IU/mL on average. HBV DNA and HBeAg were found to be 59% associated, HBV DNA and HBeAb to be 46%, and HBeAg and HBeAb to be 77%, according to the correlation study. Epidemiological insights into the distribution and correlation of HBV markers in chronic hepatitis B patients are provided by these findings.

Conclusion: This study concluded that not all chronic HBV patients have HBeAg and HBeAb levels, which are lower than those of HBV DNA. These findings offer valuable information on the serological trends of chronic hepatitis B, which might aid in comprehending how the illness develops.

Keywords: mean value; HBV DNA; HBeAg; HBeAb; chronic hepatitis B; correlation.

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Introduction

The hepatitis B virus belongs to the Hepadnaviridae family and contains the human hepatitis B virus (HBV). Liver infections brought on by HBV can be either acute or persistent. As a major contributor to cirrhosis, hepatocellular carcinoma (HCC), and chronic liver disease, HBV is a major global public health issue with significant morbidity and death rates [1, 2]. Chronic hepatitis B is characterized by inflammation of the liver caused by the hepatitis B virus (HBV) and infection that lasts for more than six months [3].

The number of chronic HBV surface antigen (HBsAg) carriers worldwide is estimated to be approximately 240 million, with considerable regional variation in the proportion of HBsAg-positive individuals between low (2%) and high (>8%) endemic levels. Improvements in socioeconomic status, large-scale vaccination campaigns, and presumably effective antiviral therapies are causing prevalence to decline in many highly endemic countries [4]. However, migration and population movements change the incidence and prevalence in low-endemic areas such as Western Europe. This is due to the higher prevalence of HBsAg among refugees and immigrants from countries outside Europe than the native European population [5].

About 60-80% of primary liver cancers in countries where HBV is widespread are caused by chronic HBV infection. In addition, it ranks third among all causes of death in Africa, Asia and the Pacific [6, 7]. Globally, HBV-related deaths from liver cirrhosis and/or hepatocellular carcinoma (HCC) increased by more than one-third between 1990 and 2013 to more than 686,000 cases per year [5]. The relationship between HBeAg, HBeAb, and HBV DNA levels in chronic hepatitis B patients is poorly understood despite a wealth of studies on the virus, particularly in Swat, Pakistan. An understanding of these interactions can enhance the monitoring and management of diseases. The Hepadna viridae family of small, enveloped, predominantly hepatotropic DNA viruses includes human HBV [8]. At 3.2 kbp, the HBV genome is partially double-stranded relaxed circular DNA (rcDNA). It consists of four open reading frames (ORFs: S-, C-, P- and X-ORF) that encode seven proteins: HBV e antigen (HBeAg, secreted dimeric protein), HBV core antigen (HBcAg, viral capsid protein), HBV polymerase/transcriptase (HBV Pol/RT, reverse protein with small reverse activity for viral glycemia and large surface protein of preglycoprotein reversion S1, PreS2, HBsAg) and HBV x antigen (HBxAg, a transcriptional regulator for the initiation of infection) [9]. Research on the measurement of HBeAg, HBeAb, and HBV DNA in chronic hepatitis B patients has mostly concentrated on larger populations; little information is known about Swat, Pakistan. Specific research is necessary to enhance local illness monitoring and management due to regional differences in immune response and disease patterns. This study fills this gap by assessing how these indicators correlate in Swat's chronic HBV patients.

HBV can spread horizontally or vertically during pregnancy. Perinatal transmission of HBV involves the presence of hepatitis B surface antigen (HBsAg) or HBV DNA within the first 6–12 months after birth in an infant delivered to an infected mother [10, 11]. Incubation of acute hepatitis B takes two to three months [12]. This is followed by a brief prehistoric period that includes nonspecific symptoms, including fever, exhaustion, loss of appetite, nausea, and body aches. Serum ALT levels rise during this phase, and HBsAg and HBV DNA can be found. The icteric phase with jaundice occurs after the prehistoric period and lasts several days [1]. The severity of the disease affects the prognosis of patients with chronic hepatitis. Five-year survival is approximately 50% in advanced patients with severe chronic hepatitis and cirrhosis [1].

According to the presence or amount of HBeAg, HBV DNA, liver transaminases (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]), and the existence of histological evidence of hepatic necroinflammation or fibrosis, chronic hepatitis B infection can be divided into four stages. Not all patients go through every stage of chronic HBV infection, and continuous phase regression is conceivable. Age of infection has a significant effect on clinical outcomes [13, 14].

Research Problem

A significant public health concern is hepatitis B virus (HBV) infection, especially in endemic areas like Pakistan's Swat. A significant contributor to the consequences of liver disease, such as cirrhosis and hepatocellular carcinoma, is chronic hepatitis B (CHB). Hepatitis B e Antigen (HBeAg), Hepatitis B e Antibody (HBeAb), and HBV DNA levels are among the virological and serological indicators that impact the course of the disease. However, it is yet unknown how these markers relate to one another in the local population, necessitating more research.

Research Focus

This study aims to better understand the immune response and disease progression in chronic hepatitis B patients by investigating the link between HBV DNA, HBeAg, and HBeAb levels. This evaluation may aid in improved disease monitoring and management.

Research Aim and Research Questions

The primary aim of this study is to measure the levels of HBV DNA, HBeAg, and HBeAb in chronic hepatitis B patients in Swat and ascertain how they correlate. The objective of the study is the following.

1. Measure and compare the amounts of HBeAg, HBeAb, and HBV DNA in the serum of patients with chronic hepatitis B
2. Examine the relationship between these markers to comprehend how they affect the course of the disease
3. Offer information that could help with better clinical decision-making and disease monitoring.

Case Presentation

The study was conducted from December 2023 to April 2024 in the Molecular Biology laboratory at Saidu Teaching Hospital Swat, Khyber Pakhtunkhwa, Pakistan. After proper permission from the hospital's directors, about 100 blood samples were obtained from chronic hepatitis B patients from different tertiary care units and rural health centers. Blood samples were taken from the patient using a sterile syringe and standard phlebotomy techniques and stored in gel tubes for further processing. Informed consent was obtained from patients on the pre-designed proforma when sampling and collecting clinical information. All the relevant clinical information was recorded while filling the proforma during blood sampling.100 blood samples stored in gel tubes were centrifuged at 4000 rpm for 10 minutes to separate the serum from the whole blood. The collected samples were run on Real-time PCR to confirm the DNA of the hepatitis B virus.

Data were statistically analyzed through Microsoft Excel and SPSS (17.0 edition) software. The Prevalence and Mean were calculated for HBeAg and HBeAb.

DNA extraction was extracted using (Promoter) automatic extraction analyzer and (Tanbead) extraction kits. This methodology was based on fully automated extraction by using magnetic beads methodology. There are 6 vials for 1 sample extraction; in the first vial, samples were added; in the second vial, lyse buffer is present for sample lysis; in the third vial, wash buffer 1 is present, in fourth vial, wash buffer 2 is present, in fifth vial elusion buffer is present, while in last sixth vial RNA/DNA Extracts were present after successful automated extraction in 30 minutes of duration.

Reagents kits used for master mix preparation provided by (Saccace Biotechnologies). The reagents kit contains one tube of 0.2ml (96 tests) ready-to-use reagent containing buffer solution and enzyme mix. These master mix tubes (Taq Polymerase enzyme, Primers, probes, and other reagents) were mixed in an Eppendorf tube using a vertex machine to mix well in a laminar flow hood to avoid contamination in the reaction. After mixing well, this mixer was added to 96 reaction tubes simultaneously. After that, (50 microliters) of RNA/DNA extracts were added to the first 94 reaction tubes, and (50 microliters) of external negative and positive control were added to the 2 last reaction tubes for further processing [15].

The amplification was done on a fully automated thermocycler analyzer (BIORAD-CFX96) by entering the amplification cycles and steps provided in reagents amplification kits. The first step lasts for 15 minutes at 95C0, named the hold stage, and the second step, named cycling 1, is divided into 3 stages: stage 1 lasts for 5 seconds at 95C0, stage 2 lasts for 20 seconds at 60C0, stage 3 lasts for 15 seconds at 72C0. The third step, cycling 2, is divided into 3 stages: stage 1 lasts for 5 seconds at 95C0, stage 2 lasts for 30 seconds at 60C0, and stage 3 lasts for 15 seconds at 72C0 for 40 cycles. In this step, the detection camera was ON to detect the hepatitis B virus [16].

The reagent kit detected HBV DNA using HEX (yellow channel) and FAM (green channel) for internal control (IC). An S-shaped amplification curve in the FAM and HEX channels showed a favorable outcome. A test for HBV DNA was deemed harmful if only the FAM channel displayed an S-shaped curve. The conclusion was inconclusive if the FAM and HEX curves were missing (flat), suggesting there may have been problems with sample processing. To ensure appropriate sample preparation in these situations, DNA/RNA extraction was carried out again. The cut-off value for detection of viral load was less than 25 IU/mL; this means that the amplification curve/viral load below 25 IU/mL was marked as Detected/Positive for HBV, and the amplification curve/viral load above 25 IU/mL were marked as Non-detected/Negative for HBV.

Determination of Hbe Antigen & Hbe antibodies

CLIA (chemiluminescence immune assay) was used to determine the Hbe antigen and Hbe antibodies in samples. Cobas e601 automated analyzer was used to detect antigens/antibodies using Elecsys HBeAg and Elecsys HBeAb reagent kits [17].

First, the analyzer was calibrated using the calibrator (HBeAg CAL1 and HBeAg CAL2) provided by the reagents kit manufacturer. After successful calibration, the high, low and normal controls were run to confirm the calibration

results. After that, a total of 100 blood samples were added to serum cups and kept these samples in a CLIA analyzer (Cobas e601) for data entry of each sample simultaneously. Then, the sample START button was pressed on the analyzer; after successful completion of the process, the results were shown on the screen of the analyzer. The cutoff value for the HBeAg is 1.0 U/mL; if the sample value was below 1.0 U/mL, the result was marked as negative; if the sample value was above 1.0 U/mL, the result was marked as positive [18].

First, the analyzer was calibrated using the calibrator (HBeAb CAL1 and HBeAb CAL2) provided by the reagents kit manufacturer. After successful calibration, the high, low and normal controls were run to confirm the calibration result. After that, a total of 100 blood samples were added to serum cups and kept these samples in a CLIA analyzer (Cobas e601) for data entry of each sample simultaneously. Then, the sample START button was pressed on the analyzer; after successful completion of the process, the results were shown on the screen of the analyzer. The cutoff value for the HBeAb is 1.0 U/mL; if the sample value is below 1.0 U/mL, the result is marked as negative; if the sample value is above 1.0 U/mL, the result is marked as positive [19].

Quantification of HBV DNA

100 blood samples were examined for chronic HBV patients on Real-time PCR (qPCR) based detection. All these 100 samples were positive for HBV virus with a viral load of (Mean=60,800). According to gender-based detection, 55 (55%) samples were collected from males, and 45 (45%) samples were collected from females. All Male patients were positive for HBV with a viral load of (Mean=62,100), and all Female patients were positive for HBV with a viral load of (Mean=58,700) see Figure 1.

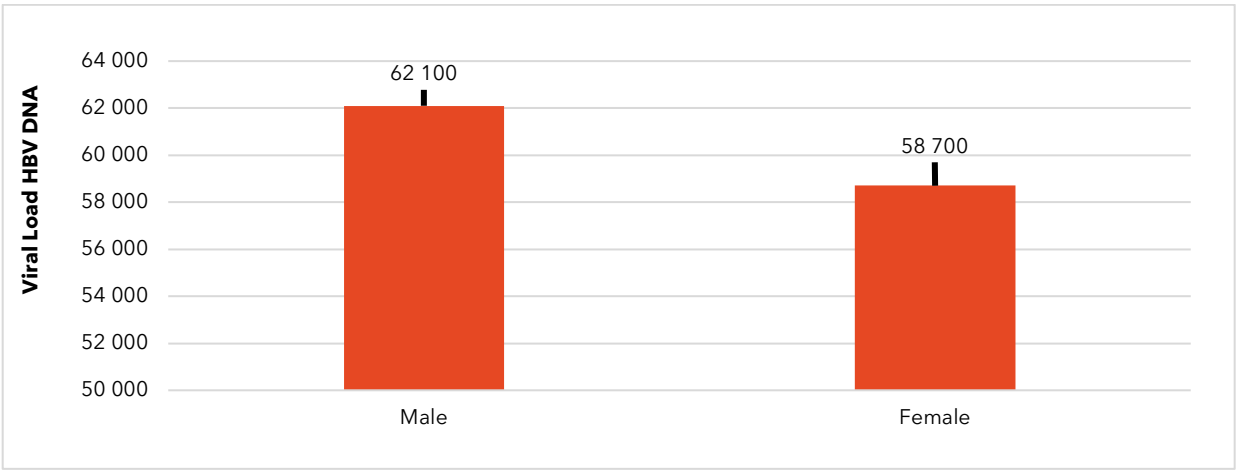

Figure 1. Gender Wise distribution of Chronic HBV DNA

Quantification of HBe Antigen

One hundred blood samples were examined for HBe antigen on CLIA methodology-based detection. In 100 samples, 57 (57%) were positive for HBeAg with a mean value of (M=12.82). According to Gender Antigen detection, 55 (55%) blood samples were collected from Males, and 45 (45%) samples were collected from males. Out of 100 samples, 28 (28%) Male patients were positive for HBeAg with a mean value of (M=11.43), and 29 (29%) Female patients were positive for HBeAg with a mean value of (M=14.52) see Figure 2.

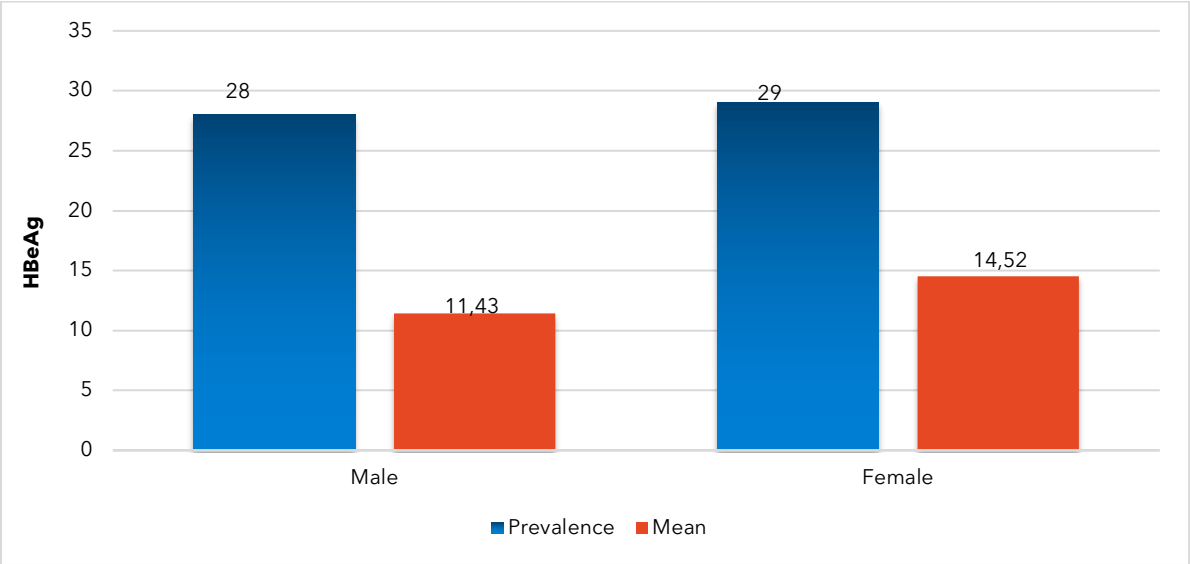

Figure 2. Gender Wise distribution of HBeAg

Quantification of HBe Antibodies

100 blood samples were examined for HBe antibodies using CLIA methodology-based detection. In 100 samples, 47 (47%) were positive for HBeAb with a mean value of (M=8.75). According to Gender-based Antibody detection, 55 (55%) blood samples were collected from Males, and 45 (45%) samples were collected from females. Out of 100 samples, 21 (21%) Male patients were positive for HBeAb with a mean value of (M=7.80), and 26 (26%) Female patients were positive for HBeAb with a mean value of (M=9.90) see Figure 3.

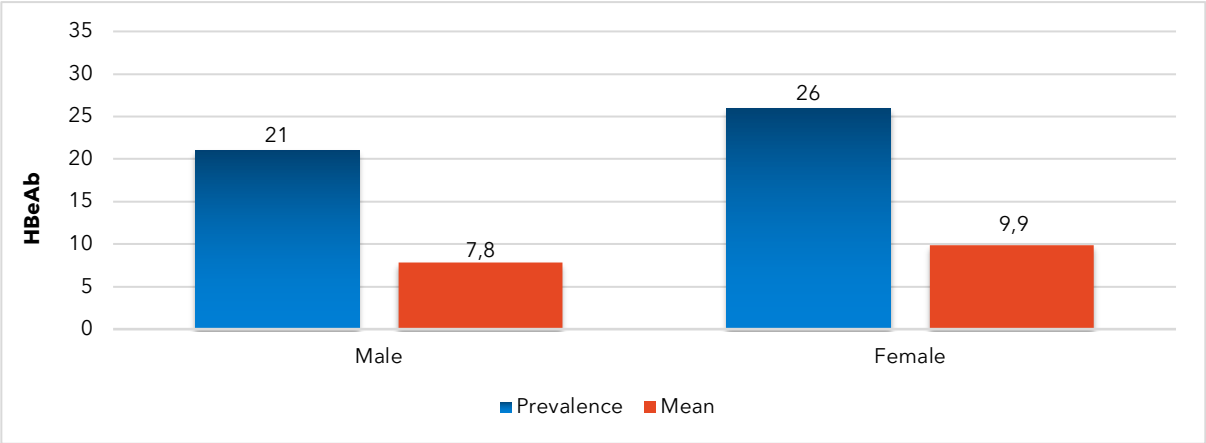

Figure 3. Gender Wise distribution of HBeAb

Correlation between HBV DNA, HBeAg and HbeAb

The results showed that the correlation between (HBV DNA-HBV DNA, HBeAg-HBeAg and HBeAb-HBeAb) was 1.0, which means 100 % correlation. The correlation between (HBV DNA-HBeAg) was 0.59, which means the correlation was 59 %. The correlation between (HBV DNA-HBeAb) was 0.46, which means the correlation was 46 %. The correlation between (HBeAg-HBeAb) was 0.77, which means the correlation was 77 % (see Table 1).

Table 1. Correlation Between HBV DNA, HBeAg and HBeAb

	qRT-PCR	HBV Ag	HBV Ab
qRT-PCR	1.00	0.59	0.46
HBV Ag	0.59	1.00	0.77
HBV Ab	0.46	0.77	1.00

Discussion

In clinical practice, blood samples are frequently used to identify HBV indicators because they provide a convenient, affordable, and non-invasive substitute for liver biopsies. Although biopsy samples offer insight into liver damage and viral replication, they are intrusive, uncomfortable, and not often performed on all patients [20]. On the other hand, serum-based testing makes it possible to regularly check the levels of HBV DNA, HBeAg, and HBeAb, which makes it more useful for managing and diagnosing the condition. High sensitivity and specificity in identifying HBV markers in blood samples are guaranteed by using qRT-PCR and CLIA in this investigation. The results immediately apply to ordinary patient treatment because these techniques are common in clinical settings. As a result, blood sample collection is still a legitimate and clinically relevant method of diagnosing chronic hepatitis B.

The use of qRT-PCR and CLIA in this study ensures reliable detection. These techniques have been validated for their accuracy in measuring viral load and serological markers in blood samples [21]. Therefore, the results remain valid because they reflect real-world diagnostic conditions commonly used in hepatitis B patient management. Despite these differences, serum-based testing is clinically relevant because it offers a practical, non-invasive, reproducible method for assessing HBV infection.

The clinical course of chronic hepatitis B is unpredictable and variable. HBeAg, HBsAg, and HBV DNA are often detectable during the early stages of infection; however, serum transaminases are typically only slightly too moderately elevated during this time. The severity of the disease affects the prognosis of patients with chronic hepatitis. Five-year survival is approximately 50% in advanced patients with severe chronic hepatitis and cirrhosis [22]. Blood samples from our investigation had greater amounts of HBV DNA than HBeAg and HBeAb. We obtained contrasting results Using liver biopsy samples: 54.3% of patients had anti-HBe, and 46.9% of patients were positive HBeAg. This discrepancy could arise because blood tests show circulating markers, but biopsy finds viral activity directly in the liver. Blood-based qRT-PCR and CLIA are realistic and efficient substitutes for routine HBV monitoring since, even though biopsy offers more accurate data, it is an intrusive procedure that is not frequently carried out [23]. Compared to patients who were anti-HBe positive, patients with HBeAg had a significantly lower mean age and a higher mean HBV DNA value. This study showed agreement with our study, but samples taken from patients were much more different in both studies. In our study, we used blood samples from chronic hepatitis B-positive patients, while in another study, we used biopsy samples from chronic hepatitis patients. The positivity, sensitivity, and specificity of liver biopsy samples were higher than those of blood samples. Studies employing liver biopsy samples typically report higher sensitivity for HBV DNA, HBeAg, and HBeAb detection in direct detection of viral replication within liver tissues. In contrast, as employed in this study, serum-based detection may have slightly lower sensitivity but is still a commonly used and less invasive method for diagnosing and tracking chronic hepatitis B [24]. The methodology used in this study has more specificity and sensitivity than other technologies. To check the sensitivity and specificity of this method, a study was conducted using Chemiluminescence immunoassay (CLIA) and highly sensitive immunoassays automated analyzers based on electrochemiluminescence technology. ECLIA and ELISA were found to be highly concordant for HBsAg, (92.62%) concordant for anti-HBs, (100%) concordance for HBeAg, (76.75%) concordance for anti-HBe and (58.67%) concordance for anti-HBc. In patients with both HBeAg and anti-HBe concomitant, the concordance for HBeAg detection was (45.83%), and the concordance for anti-HBe detection was (79.17%). Test discrepancies were primarily related to variations in sensitivity [17].

The antigen and antibody levels in chronic hepatitis patients were high in females and age group 31 to 60 years. As compared to the study conducted in which 97 patients were investigated, results showed that the mean age of the 97 patients was 39 ± 11 years, with 70 (72%) male patients and 27 (28%) female patients [21]. The HBeAg status of 87% was negative. HBV DNA levels and HbsAg titer were shown to be substantially different between HBeAg-positive and negative patients by the Mann-Whitney test ($P = 0.001$ and $P = 0.009$, respectively). According to the Spearman test, there was no discernible relationship between the levels of HBsAg and HBV DNA ($P= 0.606$ and $r = 0.53$). The methodology used in our study is different from the other study, which may impact frequency and results of antigen, antibodies and detection of HBV DNA in chronic hepatitis B patients [25].

Conclusion

According to this study, not all patients with chronic hepatitis B showed detectable levels of HBeAg or HBeAb, and HBV DNA levels are greater than those of these antibodies. These results emphasize how crucial HBV DNA testing is for improved disease surveillance, particularly in individuals with negative HBeAg results. According to the findings, blood-based qRT-PCR and CLIA tests are trustworthy, non-invasive substitutes for liver biopsies in regular diagnosis and treatment. To enhance treatment choices, this study recommends that HBV DNA testing be given priority in clinical procedures. Additionally, future studies should examine how these markers evolve.

Suggestions for Future Research

The research results present multiple areas of investigation that would advance scientific understanding and medical care for chronic hepatitis B (CHB). Time-dependent investigations need to follow HBV DNA HBeAg and HBeAb alterations to reveal disease progression patterns and how HBeAg changes into HBeAb. The evaluation of biomarkers that can forecast clinical results, such as cirrhosis and hepatocellular carcinoma (HCC), needs further research. Researchers should examine how hereditary host elements and immune processes affect different marker correlations. The variation in immune responses between different populations, especially in Swat Pakistan, explains why their HBV infection displays diverse clinical manifestations. Scientists should investigate genetic variations and immunological elements that control HBV replication and antigen manifestation.

The clinical use of next-generation sequencing and digital PCR methods should be investigated because they would enhance the detection of discreet viral levels and cryptic infections. These monitoring tools would support existing qRT-PCR and CLIA methods through better precision measurement capabilities. Studies must explore the effects that antiviral therapy has on the relationships between HBV markers. Clinical assessments between treatment groups and non-treatment controls will help doctors understand treatment effects on the relationships among HBV DNA, HBeAg, and HBeAb, which could optimize individual patient treatment approaches. The advancement of CHB treatment requires research that evaluates the effectiveness of RNA interference and immune modulator therapies.

Declarations

Author Contributions

Conceptualization, H.A. and M.R.; methodology, H.A.; software, Q.A.; validation, H.A., M.R. and A.A.; formal analysis, Q.A.; investigation, H.A.; data curation, I.U.; writing—original draft preparation, H.A.; writing—review and editing, M.R. and A.A.; visualization, N.A.; supervision, A.A.; project administration, M.R.; funding acquisition, N.A. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declares that there is no conflict of interests regarding the publication of this manuscript.

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