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Molecular Detection of Precore and Basal Core Promoter Regions of Hepatitis B Virus by Using Nested Polymerase Chain Reaction

Zahraa Abdul Ameer Atiyah[1], Dhuha Mahdi Jabir[2]

[12] Department of Biology, College of Science, University of Al-Qadisiyah, Al-Diwaniyah, Iraq

Corresponding author: Dhuha Mahdi Jabir; Email: dhuha.mahdijabir@qu.edu.iq

Email: sci.bio.mas.25.14@qu.edu.iq

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Abstract

Background: Hepatitis B virus (HBV) is a significant health care concern in the global context, given that it is among the prevalent causes of chronic liver diseases, including cirrhosis and hepatocellular carcinoma. The description of the viral activity and the investigation of its molecular peculiarities are significant to identify the regions that are crucial regulators of the HBV genome.

Purpose: To identify the precore and basal core promoter (BCP) of HBV by application of the nested polymerase chain reaction (nested PCR).

Methods: The blood bank and Diwaniyah General hospital in Diwaniyah, Iraq provided 20 of serum samples that are known to have been infected with HBV. Nested PCR amplification of the precore and basal core promoter regions of the viral DNA was done with specific outer and inner primers. The amplification products were confirmed using Agarose gel electrophoresis to ensure that amplification was successful.

word results: The molecular detection of the target areas was successful as all the 20 samples (100%) had a clear and distinct DNA band at the expected amplification position.

Conclusions: These results suggest that nest polymerase chain reaction (nested PCR) is a sensitive and valid method used to identify and monitor HBV infection in a water with ease especially to determine the precore and basal core promoter areas in the clinical serums.

Keywords

Hepatitis B virus; Nested PCR; Precore region; Basal core promoter; Molecular diagnosis

Introduction

Hepatitis B virus (HBV) is a severe issue of the world population and a primary cause of chronic liver disease, such as cirrhosis and hepatocellular carcinoma [1,2]. HBV continues to prevail in most of the developing nations or countries because of the failure to control the spread of the infection and the absence of proper healthcare services despite the efficient vaccines [1,3].

Viral replication activity and the host immune response are also complicated and they determine the clinical outcome of chronic infection of HBV that varies substantially among individuals who have the infection [4,5]. All these are necessitated to identify the future of the disease and the long-term effects [6]. Hepatitis B virus family (Hepadnaviridae) which comprises the partially double-stranded DNA viruses is defined by a unique replication cycle through reverse transcription of pregenomic RNA [7]. The genetic variation enables the genetic variation of viruses and results in numerous viral population [4].

The persistence of hepatitis B virus (HBV) is influenced by viral genetic diversity, which influences the resistance of hepatitis B virus to the immune system and the efficacy of antiviral treatment [8,9]. In such a way, the molecular identification of regulatory areas in HBV virus genome is critical to the comprehension of the pathogenicity and disease prognosis of the virus [10].

HBV BCP (basal core promoter) is a crucial regulation factor that regulates viral replication and transcription [10,11]. The expression of precore and pregenomic regions as messenger RNA (mRNA) is predominantly regulated, and it was found to significantly influence the viral replication and HBeAg expression [12].

As the virus is actively replicating, mutations can be developed in the precore and basal core promoter regions which can lead to minimal or total production of HBeAg. Such changes are directly linked to the rising rates of chronic infection of hepatitis B virus (HBV) of the HBeAg-negative type in most geographic areas and are associated with the evasion of immunity and disease progression [13,14].

The molecular epidemiology and molecular pathogenesis of hepatitis B virus requires extensive molecular studies on the precore and basal core promoter regions. These genetic studies can be used in the better characterization of diseases because the modifications of these regions can affect the rate of viral replication and clinical presentation [15]. The high-sensitivity nested PCR is used to characterize and amplify low-abundance viral DNA, which is typically applied in clinical samples with low viral loads [16,17]. This approach is also most effective at specifically locating regulatory sequences present in the HBV genome such as the precore and basal core promoter regions because it leads to increased specificity and decreases

nonspecific amplification [18]. It has been shown that nested PCR protocols are more sensitive than other PCR-based methods, particularly, in the detection of low-copy HBV DNA in clinical samples [17]. Thus, the present study used nested PCR to identify the molecular characteristics of precore and basal core promoter of HBV and to offer a dependable molecular data of the presence of the two in serum samples.

Materials and Methods

Study design

According to the observations, the cross-sectional study applied nested polymerase chain reaction (nested PCR) to identify the basal core promoter and regulatory regions of hepatitis B virus (HBV) at molecular level. The study was conducted in the Molecular Biology Laboratory, College of Science, University of Al-Qadisiyah, Diwaniyah, Iraq, from August to December 2025, using twenty HBV-positive serum samples to evaluate nested PCR.

Study setting

Molecular analyses were done in the Molecular Biology Laboratory, College of Science, University of Al-Qadisiyah, Al-Diwaniyah, Iraq.

Sample collection

The study included 20 serum samples positive for HBV collected from the blood bank and Al-Diwaniyah General Hospital. Samples were transferred to the molecular laboratory where HBV positivity was confirmed.

Inclusion and exclusion aspects

HBV-positive serum samples were included. Hemolyzed samples or those with insufficient volume were excluded.

Ethical considerations

Ethical approval was obtained from the Ethics Committee of the Diwaniyah Health Directorate (374). Informed consent was obtained, and samples were handled with strict confidentiality.

DNA extraction

Viral DNA was extracted using a commercial kit according to the manufacturer's instructions and stored for nested PCR amplification of precore and BCP regions.

PCR reaction mixture

A commercial PCR master mix was used with a total volume of 25 µL, including primers, template DNA, and nuclease-free water. The second PCR used the first-round product as template. All reactions were performed under sterile conditions.

Nested PCR amplification

Nested PCR amplification of precore and BCP regions was performed. The first round used primers BCP1-F and BCP1-R with initial denaturation at 94°C for 5 min, followed by 30 cycles (94°C 1 min, 55°C 30 sec, 72°C 1 min) and final extension at 72°C for 10 min.
The second round used primers BCP2-F and BCP2-R with conditions: 94°C 5 min, 30 cycles (94°C 45 sec, 52°C 30 sec, 72°C 1 min) and final extension at 72°C for 10 min.

Agarose gel electrophoresis

PCR products were analyzed on 1.5% agarose gel in TAE buffer, stained with nucleic acid stain, with DNA ladder, and visualized under UV light.

Results and Discussion

The precore and basal core promoter region of the hepatitis B virus (HBV) was molecularly identified through the use of serum samples with the help of the nested polymerase chain reaction (nested PCR). The 20 of 20 serum samples (100%) were all positive and clear and distinct DNA bands were formed on agarose gel electrophoresis at the desired amplification size. The efficiency of the primer sets and the optimality of the thermal cycling conditions that were aimed at the precore and basal core promoter regions of the HBV genome was reflected in the sharp and specific amplification of the bands. There was no non-specific amplification or background staining, which implied that the nested PCR assay was highly specific.

These results prove that nested PCR is a sensitive and faithful method of identifying HBV precore and basal core promoter regions in clinical serum specimen (Figure 1) [19].

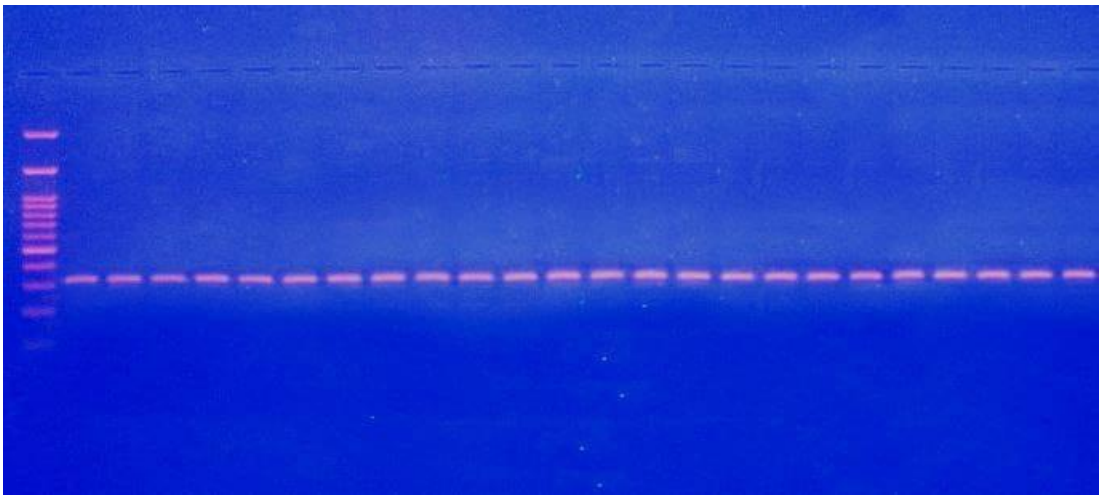

Figure 1. Nested PCR product of precore and basal core promoter regions of hepatitis B virus (HBV) electrophoresis on agarose gel. The amplification product was 467bps in the first round PCR and 316bps in the second round (nested) PCR. Amplified fragments were run on agarose gel 1.5 percent, stained using nucleic acid dye, and examined using ultraviolet lighting at 302nm. Lane M gives the DNA

molecular weight marker and lane 1-20 gives the amplified HBV precore/BCP fragments.

These findings prove the biological significance of the emphasis on precore and basal core promoter regions of the HBV genome and validate the technical possibility of the nested PCR. Mutations in these regions are linked to alterations in the expression of HBeAg and changes in the dynamics of viral replication, and these areas are believed to be some of the major regulatory factors of viral replication and transcription [10,20]. Thus, the molecular characterization of the precore and basal core promoter region, which may not be accompanied by any sequencing analysis, may be incredibly helpful in providing information about viral activity and persistence [8,18]. The amplification of these regions was successful in the current study, which justified the use of these molecular targets to detect hepatitis B virus (HBV), and emphasized the relevance of these molecular and clinical targets in the study of chronic HBV infection [13].

This article agrees with the existing molecular research which has indicated that nested polymerase chain reaction (nested PCR) is a powerful method of HBV DNA detection especially when the regulatory sequences of the virus including the precore and basal core promoter sequences are to be target. A number of studies demonstrated that the nested PCR approach has a greater sensitivity and specificity than the traditional polymerase chain reaction (PCR) techniques and can detect the hepatitis B virus (HBV) in clinical samples of low viral load [19,21].

The amplification outcomes are also similar to the previous findings that showed the success of HBV detection by amplifying the precore and basal core promoter regions

with the application of nested PCR and the use of agarose gel electrophoresis [18,22]. This consistency can justify the use of nested PCR as an effective diagnostic and molecular surveillance method of HBV infection. Although the nested PCR could be used to achieve molecular identification of the HBV precore and basal core promoter regions, there are certain limitations to be taken into account in the research. It was not entirely clear as the genetic variations of the amplified regions were not determined by genomic sequencing analysis, the sample size was rather small, and it may restrict the external validity of the results [20,23]. However, these shortcomings do not disregard the central objective of the research which was the attainment of trustworthy molecular detection of precore and basal core promoter areas of hepatitis B virus (HBV) and gives a possibility to conduct further research with more sample sizes and detailed sequencing.

Conclusion

Overall, the study showed that nested PCR can be used in the identification of the precore and basal core promoter regions of the hepatitis B virus (HBV). Agarose gel electrophoresis confirmed the sensitivity and specificity of the method through clear amplification patterns. These results indicate that nested PCR is a reliable technique for detecting important regulatory regions of the HBV genome in clinical serum samples. However, sequencing analysis was not included, and further studies are required for more detailed molecular characterization.

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