Identification of hepatitis B virus core mutants by PCR-RFLP in chronic hepatitis B patients from Punjab, Pakistan

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Summary

Chronic hepatitis B virus (HBV) infection remains a major health issue worldwide. Several factors including core gene variation are responsible for the development of chronicity of HBV infection. The present study was designed to identify the variations in the core region of the HBV genome in a local population of chronic hepatitis B patients (n = 57) using a PCR-based restriction fragment length polymorphism (PCR-RFLP) method. Fifty subjects were found to be positive for the presence of HBV DNA. For the core region genotyping, the Ava II and Msp I restriction enzymes were used. Mutations at nucleotide (nt) 2147 and nt 2362 in the HBV genome in the core region for Ava II (A4 type, 74%) and nt 2331 for Msp I (M1 type, 66%) were observed as the most common pattern. These results are different from those of previously reported studies on other populations and thus appear to be unique to the Pakistani population. This type of characterization of core mutants may be useful for the design of vaccines based on viral epitopes that are effective for the Pakistani population. Moreover, these unique genotypic patterns for the HBV core gene might be some of the main factors responsible for understanding the underlying mechanism by which HBV chronicity is developed in the Pakistani population.

Abbreviations

CTL cytotoxic T lymphocyte
MHC major histocompatibility complex
PCR polymerase chain reaction
RFLP restriction fragment length polymorphism

Introduction

Hepatitis B virus (HBV) is one of the causes of the development of chronic liver diseases and is an etiological agent of both acute and chronic viral hepatitis. HBV is a blood-borne pathogen and can be transmitted parenterally, sexually, perinatally or by saliva [11].

HBV infection is a global health problem, and current estimates show that about 2 billion people are infected with HBV worldwide, and 350 to 400 million of them suffer from chronic HBV infection [24]. Approximately 0.5 to 1 million people die
every year from HBV-related liver diseases [13]. In Pakistan alone, the HBV prevalence rate is 5% and thus affects 7 million people [1]. Patients with chronic HBV infection are at higher risk of developing chronic liver diseases, including liver cirrhosis and hepatocellular carcinoma [13], and nearly 25% are terminated in untreatable liver cancer [18].

Several host-related factors are associated with an increased risk of developing chronic infection [9, 16]. Thus, the course of infection depends on the host immune response, age at infection and host genetic factors (e.g. human leukocyte antigen, HLA) [12]. HBV sequence variability has also been increasingly recognized as a factor that modulates the course and outcome of HBV infection [23]. The role of MHC II (major histocompatibility complex II) polymorphism in the outcome of HBV infection has also been investigated in a number of studies [3, 22], since the proper antigen presentation with the help of MHC II to the helper T cells is important for HBV clearance [22].

HBV has the smallest genome of all replication-competent animal DNA viruses [13]. The HBV genome consists of an about 3-kb-long partially double-stranded DNA [18] with four open reading frames (ORFs). These ORFs encode; the envelope (preS1, preS2 and HBsAg), the core (hepatitis B core antigen, HBcAg; hepatitis B e antigen, HBeAg), the X protein and the polymerase [11]. The four protein-coding regions (the ORFs) are translated into seven known proteins; among these, the HBcAg is crucial for developing chronicity in HBV-infected patients. HBcAg (21-kDa protein) is an integral part of the nucleocapsid, with a basic C terminus that is essential for the packaging of viral nucleic acid. After being transcribed, the HBcAg, along with HBeAg, is targeted to the endoplasmic reticulum, where HBcAg is cleaved and HBeAg is secreted. The HBeAg (16–18 kDa protein) is antigenically distinct from HBcAg. The HBeAg is a soluble protein that can be detected in the serum of patients with high virus titers; however, it is not essential for viral replication [13].

The cellular and humoral immune responses to HBV infection are quite complex, and HBcAg is regarded to play a role in the development of chronic infection. It has been suggested that the cellular response to several viral proteins correlate with the severity of clinical disease and viral clearance [5]. In this respect, different epitopes within core protein show differences in their immunological aspects [11].

The HBcAg and HBeAg present themselves as potential targets for antiviral immunity as they share a large number of amino acids and some epitopes for B and T cells [2], and both of these antigens are highly cross-reactive at the T-cell level.

The HBeAg therefore presents immunologic epitopes to the T cells, thus protecting the HBcAg-expressing hepatocytes against the immune response. The main determinant of whether a person clears the infection or becomes a chronic carrier is the adequacy of the cytotoxic T cell (CTL) response. During the asymptomatic period of infection, the viral replication is reduced by CD8+ CTL, whose target is the HBcAg.

Mutations in the core gene are found to provoke severe chronic liver diseases. Variations inside the CTL epitopes of the core protein therefore might create immune escape mutants, leading to chronic viral persistence and severe liver disease [11]. Such mutations have already been identified by sequencing the cloned PCR products. Detection and characterization of new variants of the core gene from a larger number of chronic hepatitis B patients during the course of the infection require a very rapid, specific, sensitive and simple method.

The present study has been conducted to identify the core gene mutants in chronic HBV infection. In this study, viral sequences for the C gene between nucleotides (nt) 1902 and 2454 were analyzed using the PCR-RFLP method. Detection of variants was carried out with the help of a pair of restriction enzymes, Ava II and Msp I, as reported previously [2], and their position was confirmed by alignment of all of the known HBV sequences (NCBI GenBank, 2005) using the multiple alignment software Clustal W [6]. Three different variants (A1, A2 and A4) were detected with Ava II, and similarly, three different variants (M1, M2 and M3) were found in the case of Msp I. The A4M1 type was found to be the most frequent pattern, corresponding to one recognition site at nt 2147 and another at nt 2362 for Ava II (specific for GGA/TCC) and one