



# Mutational analysis of pre-core gene of Hepatitis B Virus

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## ABSTRACT

The research is based on the fact that different courses of Hepatitis B virus infection are predisposed by the variations in the viral genome. The Hepatitis viral genome is unique as it consists of four overlapping genes, and for the purpose of this study, the pre-core gene was selected of the four genes for mutation analysis. Viral DNA was isolated from acute viral Hepatitis B patients and was amplified by polymerase chain reaction and then separated by Agarose gel electrophoresis resulting in isolated bands of 309 bps and the mutation was detected by direct sequencing. Genotyping was carried out with the help of MultAlin software, and the observations resulted in the conclusion of definite mutation in codon 17 of valine to phenylalanine, a stop codon mutation and also in codon 11 of phenylalanine to leucine of less significance (genotypic variation), thus indicating that the gene has undergone mutation at a very early stage in the course of the disease.

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## Introduction

Viral hepatitis is one of the most common infectious diseases, causing an estimated 1.5 million deaths worldwide each year.<sup>3</sup> Hepatitis B shows acute as well as chronic symptoms which result mostly in deaths but apart from this Hepatitis B can also be passed from one generation to another through people known as carriers. These carriers can transmit Hepatitis B via sexual transmission or blood transfusion.<sup>1,2</sup>

Hepatitis B virus (HBV) is a 42nm DNA virus with an outer envelope and an inner core enclosing the viral genome and a DNA polymerase.<sup>2</sup> One of the DNA strands (the plus strand) is incomplete, so that the DNA appears partially double stranded and partially single stranded. Associated with the plus strand is a viral DNA polymerase, which has both DNA-dependent DNA polymerase and RNA-dependent reverse transcriptase functions. This polymerase can repair the gap in the plus strand and render the genome fully double stranded. The genome has a compact structure with four overlapping genes. The first gene is the S gene that encodes for the surface antigen. The second gene is the P gene and codes for the DNA polymerase enzyme. The third gene is the X gene that encodes for a small non particulate protein. The fourth gene is the C gene which consists that has two regions; C and Pre-C. When the C region alone is translated, the core antigen is formed, which is assembled as the nucleocapsid core particles. It is not secreted and does not circulate in blood but produces both IgM and IgG. The IgG antibody to the core antigen persists in blood long after all other serological markers have disappeared and so provides a useful marker of prior infection with HBV.<sup>4</sup> If translation begins from the Pre-C region, the resulting protein is HBeAg, a non-particulate soluble antigen possessing a signal protein which enables it to be secreted. It is therefore present in circulation. The presence of HBeAg in blood provides a convenient and readily detectable marker of HBV replication and high infectivity.<sup>7</sup> As a result the present study was undertaken to detect markers for early detection of Hepatitis B.

## Materials and Methods

The serum was collected from patients (aged 18-35) suffering from acute hepatitis; clinically the course of acute hepatitis varies widely from mild symptoms requiring no treatment to fulminant hepatic failure needing liver transplantation. Acute viral hepatitis is more likely to be asymptomatic in younger people. Symptomatic individuals may present after convalescent stage of 7 to 10 days, with the total illness lasting 2 to 6 weeks.

The DNA was extracted using phenol-chloroform method with slight modifications.<sup>5</sup> The DNA extracted, was then amplified using PCR for 40 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1 min, with a 10 min extension step at 72°C in the end. Primers used for the Pre-core region were:

Forward primer: 1771-1790

5'-TATTAGGAGGCTGTAGGCAT-3'

Reverse primer: 2079-2060

5'-AGAATAGCTTGCCCTGAGTGC-3'

To separate the PCR amplified products, Agarose Gel Electrophoresis was followed using 2% Agarose gel and then the nucleotides were sequenced. The target PCR products within the agarose gel were purified for sequencing using the Perfectprep Gel cleanup Kit (Eppendorf, Wetsbuty, NY). On retrieving the sequence, it was analysed for mutation by comparing the sequence with that of the wild strain using the MultAlin software. The wild strain sequence was available in DDBJ, the sequence beginning from 1771 and 2079 (as per the primer used) of 309 bp was compared with the sequence suspected to be mutated.

## Results and Discussion

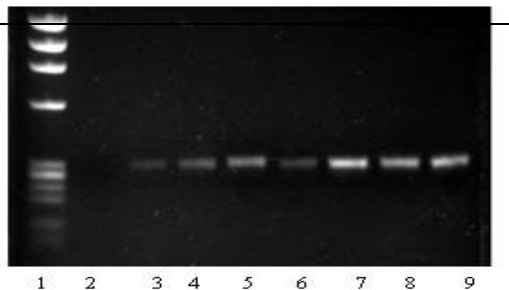
Based on direct sequencing of all the PCR products obtained we observed that in the acute viral hepatic patients, one mutation was at codon 17 where valine was found to be substituted with phenyl-alanine (Fig. 2), another mutation was a classic pre-core stop codon mutation (Fig. 3). Similar results were obtained in cases of fulminant hepatitis.<sup>6,8</sup> Other than these 2 mutations codon 11 had also a variation of phenylalanine to

leucine, but this is not considered as a mutation as it is a genotypic variation as described by Yim H J *et. al.*, 1995.<sup>9</sup> The pre-core stop codon mutation could be one of the reasons as to how the frequent viral breakthroughs limit the usefulness of vaccines in the treatment of chronic Hepatitis B.<sup>9</sup>

Acute viral hepatitis is more likely to be asymptomatic in younger people. Symptomatic individuals may present after convalescent stage of 7 to 10 days, with the total illness lasting 2 to 6 weeks. Initial features are of nonspecific flu-like symptoms, common to almost all acute viral infections and may include malaise, muscle and joint aches, fever, nausea or vomiting, diarrhea, and headache. More specific symptoms, which can be present in acute hepatitis from any cause, are: profound loss of appetite, aversion to smoking among smokers, dark urine, yellowing of the eyes and skin (*i.e.*, jaundice) and abdominal discomfort. Physical findings are usually minimal, apart from jaundice (33%) and tender hepatomegaly (10%). There can be occasional lymphadenopathy (5%) or splenomegaly (5%).

Thus, our experiment draws to the conclusion that the development of markers for the HBeAg may be an important detection technique for Hepatitis in the earliest stage otherwise acute hepatitis. However, the chronological relationship between the emergence of the pre-core mutation and the onset of Hepatitis requires further study.

#### Figures:



**Fig. 1: The gel analysis of all the PCR products obtained.**

Lane 1: The digested marker.

Lane 2: Negative control

Lane 3-9: PCR products of all positive HBV DNA pre-core regions.

5'GCACATGCACTTTTCCCTCTGCCTAATCATCTTTTGT  
TCA3'

5'GCACATGCACTTTTCCCTCTGCCTAATCATCTGTTGT  
TCA3'

**Fig. 2: The mutation of valine to phenylalanine at codon 17**

5' TGGGTGGCTTTGGGGCATGGACATTGAC 3'  
5' TGGGTGGCTTTGGGGCATAGACATTGAC 3'

**Fig. 3: The mutation to form a stop codon**

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