

## Prevalence and Characteristics of Basal Core Promoter Mutations in Iran and its Correlation with Acute and Chronic Hepatitis B Infection

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### Abstract

**Introduction:** Manifestations of HBV infection differ in chronic and acute phases. Therefore, identifying the determinants such as mutations has a vital role in the treatment of the disease. A dual transversion in the basal core promoter (BCP) region is common among HBV patients. Thus, the present study was conducted with the objective of determining the prevalence of basal core promoter (BCP) mutations and its correlation with the outcome of HBV infection.

**Method:** In this cross-sectional study, samples were obtained from 182 Iranian HBsAg positive patients who were admitted to the Bandar Abbas Blood Transfusion Organization in 2012 and 2013. They were screened by ELISA test using commercial kits to detect serological marker anti-HBc IgM for distinct chronic hepatitis from acute infection. Thereafter, the extracted DNA was used for determination of the BCP mutations by PCR-RFLP technique. Data analyses were performed with SPSS 12 by Mann–Whitney U test, Fisher’s exact probability test, and t-test.

**Results:** BCP mutations were observed in 15 samples (8.24%) of the study population, and serological tests determined that, among the BCP mutants, one sample (6.67%) was HBeAg positive, 14 samples (93.33%) were HBeAg negative, and four samples (2.2%) were positive for anti-HBc IgM test. Data analysis indicated a statistically significant association between BCP mutations and acute hepatitis ( $p=0.002$ ). However, no relationship was detected between the prevalences of the BCP mutations and gender of subjects ( $p>0.567$ ).

**Conclusions:** The prevalence of BCP variants was low in the south of Iran, and this mutation can lead to acute phase of viral hepatitis.

**Keywords:** Acute hepatitis B, Chronic hepatitis B, Basal core promoter, Epidemiology

### 1. Introduction

Hepatitis B infection is a universal health problem, resulting in nearly 350 million chronically infected carriers worldwide (1). The range of the manifestations of HBV infection differs in chronic and acute phases. In the chronic phase, symptoms vary from an asymptomatic state to cirrhosis and hepatocellular carcinoma. However, manifestations of acute phase range from icteric to anicteric hepatitis and in some cases fulminant hepatitis (2). Determination of this issue plays an important role in the treatment and management procedure of the disease. Thus, it is important to identify HBV-infected patients who are at a higher risk for disease progression to the chronic phase (3). Previous observations illustrated up to 90% of infants and 5%–10% of adults with acute HBV infection progress to chronic hepatitis B (4). Although lots of determinants can identify the progression and outcome of HBV infection, many surveys have demonstrated that development of infection is mainly influenced by the viral factors, including genomic variations (5). The mutations are usually observed due to the spontaneous error rate of viral reverse

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transcriptase. Based on the literature, among these mutants, variations in the basal core promoter (BCP) region of the genome may be related to the outcomes of HBV infection (6, 7). In fact, a dual transversion in the basal core promoter (BCP) region (nt 1742–1849) of the HBV genome, involving an A to T substitution at nucleotide 1762 (A1762T) and a G to A mutation at nucleotide 1764 (G1764A), is often observed in chronic hepatitis (8). However, controversy exists regarding the presence of the mutations during the acute phase. Although the majority of investigations have indicated the association between basal core promoter mutations and chronic phase of hepatitis B infection, others have described that these alternations can be found in acute hepatitis (9). The importance of determining the association between BCP mutations and HBV outcome is mainly considerable in weak populations such as old age (10), children (11), and infants (12) and also immunocompromised patients (13). In accordance with the literature, the highest transmission risk of these variants is in blood transfusion centers. On the other hand, there is a lack of documentation regarding the prevalence and characteristics of BCP mutations in Iran, especially in Bandar Abbas City. In addition, Bandar Abbas is one the main communication ports of Iran with the Persian Gulf countries. Therefore, in the present research, we assessed the prevalence and characteristics of BCP mutations in Bandar Abbas, Iran, and its correlation with the outcome of HBV infection.

## **2. Material and Methods**

### **2.1. Research design**

This single-center cross-sectional study was carried out in the Bandar Abbas Blood Transfusion Organization in 2012 and 2013. Enrolled patients provided written informed consent, and those who did not consent to participate were ruled out.

### **2.2. Sampling**

A total of 182 HBsAg positive patients admitted to the Bandar Abbas Blood Transfusion Organization in 2012 and 2013 were recruited in the study by census sampling method. Serum samples were obtained from the subjects, including 144 (79.12%) males and 38 (20.88%) females.

### **2.3. Data collection**

All patients were examined for anti-HIV (human immunodeficiency virus), anti-HCV (hepatitis C virus), and anti-HDV (hepatitis D virus) antibodies (Ab), and then the positive samples were excluded from the project. In addition, based on patient records, those who received antiviral treatment and those with recurrent hepatitis B after liver transplantation were ruled out. These patients were excluded due to the impact of treatment on serum HBV DNA levels and the possibility that antiviral therapy might have an effect on core promoter variants. The serum samples were evaluated by ELISA test using commercial kits (DIAPRO, Italy) to detect serological marker anti-HBc IgM according to the manufacturer's instructions. An anti-HBc IgM test was used to determine chronic hepatitis from acute infection. Thereafter, DNA was extracted from sera with the Roche High Pure Viral Nucleic Acid kit (Roche Molecular Biochemicals, Germany) by proteinase K and sodium dodecyl sulphate (SDS) treatment followed by phenol/chloroform/isoamyl alcohol (25/24/1) reagent and ethanol precipitation depending on the manufacturer's protocol. Thereafter, the extracted DNA was used for determination of the basal core promoter mutations by PCR-RFLP technique. Indeed, the core promoter region was amplified via PCR and then excised by specific restriction enzymes. To do the procedure, two HBV primers (Core1 and Core2) were used in the first PCR amplification. An aliquot of reaction product was then further amplified in the second reaction with primers, Core1 and Core3. The primer sequences were Core1, 5'-GCATGGAGACCACCGTGAAC-3' (sense, nt 1606-1625); Core2, 5'-GGAAAGAAGTCAGAAGGCAA-3' (antisense, nt 1974-1955); Core3, 5'-GGCAAAAAGAGAGTAAGT-3' (antisense, nt 1959-1940). A 5 µL of the resuspended DNA was added to an amplification mixture containing 2.5 µL of 10× Taq polymerase buffer, 0.5 µL of 25 mmol/L deoxyribonucleotide triphosphates, 0.75 µL MgCl<sub>2</sub>, 0.5 µL of Taq polymerase (Promega, Beijing, China), and one µmol each of primers Core1 and Core2 (total volume of 25 µL). Then, for the second cycle, a 1 µL of the first-round PCR products was added to an amplification mixture containing 5 µL of 10× Taq polymerase buffer, one µL of 25 mmol/L deoxyribonucleotide triphosphates, 1.5 µL MgCl<sub>2</sub>, 1 µL of Taq polymerase (Promega, Beijing, China), and 2 µmol each of primers Core1 and Core3 (total volume of 25 µL). The PCR profile was similar in both amplification cycles: an initial 5 min denaturation at 94°C, followed by 35 cycles of amplification including denaturation for 45 s at 94°C, annealing for 45 s at 58°C, and extension for 45 s at 72°C. Strand synthesis was completed at 72°C for 1 min. Finally, the PCR products were incubated with restriction enzyme Sau3AI that recognize 5'-GATC-3' sequence and cut the amplified segment into two fragments of 157 bp and 197 bp in length. However, in absence of PC mutation, the production was a fragment of 354 bp in length. The products were visualized by 3% agarose electrophoresis and ethidium bromide staining.

The PCR products were incubated with restriction enzymes Ava II and MboI (New England Biolabs, Inc., USA) for 16 h at 37°C in a 15 µL reaction sample according to the manufacturer’s recommendations.

**2.4. Statistical analysis**

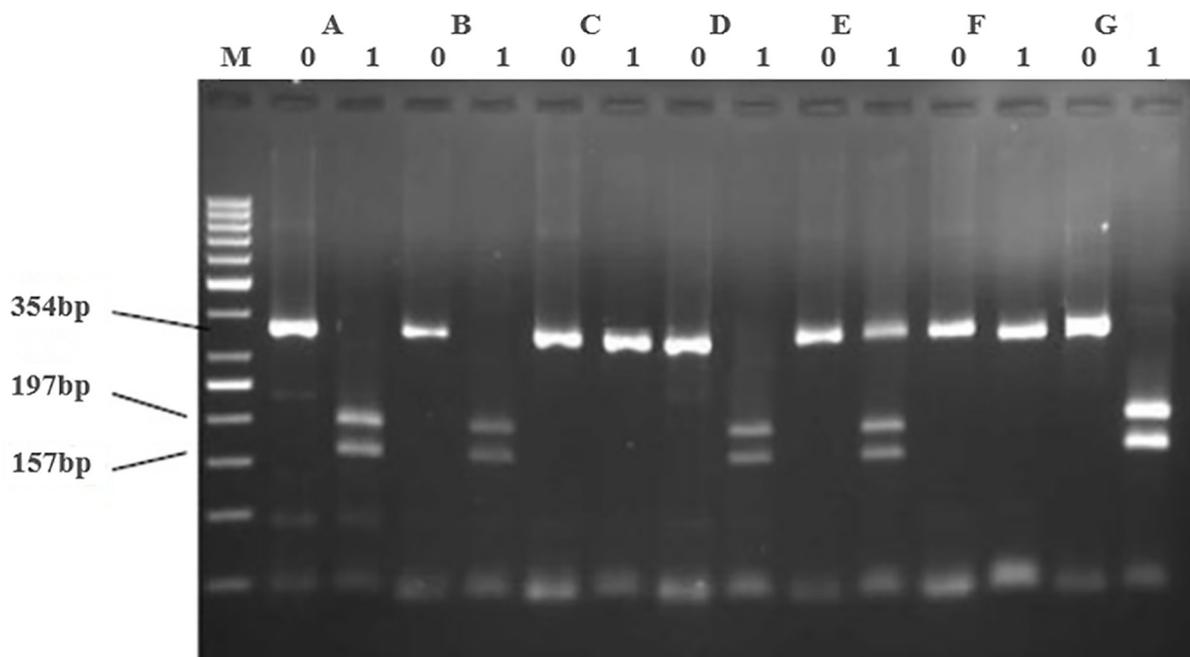
Data handling and analyses were performed with SPSS software for Windows, version 12.0, and a  $p < 0.05$  was considered statistically significant. Proportions of each factor were compared between groups using the Mann–Whitney U test and the Fisher’s exact probability test, and group means were compared using the  $t$ -test.

**3. Results**

No serum samples were excluded from the study during the research period, and all of the 182 serums were remained in present study. Thirty-eight (20.88%) were female and 144 (79.12%) were male. Basal core promoter mutations were observed in 15 samples (8.24%) of the study population, which created two fragments of 197 bp and 157 bp in length, which were detectable by electrophoresis (Figure 1). Serological tests determined that, among the basal core promoter mutants, one sample (6.67%) was HBeAg positive, 14 samples (93.33%) were HBeAg negative, and four samples (2.2%) were positive for anti-HBc IgM test. As illustrated in Table 1, among the anti-HBc IgM positive samples, two samples (50%) were the BCP mutants. Thus, data analysis indicated a statistically significant association between basal core promoter mutations and acute hepatitis ( $p=0.002$ ). Eight samples (53.33%) of the core promoter mutants were female and the others were male. Further analysis described no relationship between the prevalences of the BCP mutations and gender of subjects ( $p>0.567$ ).

**Table 1.** Analytical analysis of the study variables

Variables		Mutants	Wild types	<i>p</i> -value
Gender	Male	14 (7.7%)	62 (34.1%)	0.567
	Female	19 (10.4%)	87 (47.8%)	
Outcome	Acute	2 (50%)	2 (50%)	0.002
	Chronic	13 (7.3%)	165 (92.7%)	



**Figure 1.** Representative patterns on agarose gel electrophoresis demonstrating the restriction enzyme–based detection of basal core promoter mutants and variants. Each analysis is represented by two lanes: (1) the left without and (2) the right with a restriction enzyme Sau3AI added. C and F show the wild-type (354bp): A, B, D, and G show mutants (157 bp, 197 bp). E shows a mixture of wild types and mutants. M: Marker 50 bp.

#### 4. Discussion

HBeAg is derived from the precore and core gene of Hepatitis B virus, and mutations of which can lead to changes in the sequence, structure, antigenicity, and also the biological functions of HBeAg (14). In accordance to this literature, we found that the majority of BCP mutants (93.33%) were HBeAg negative in the present project. Lots of previous studies reported this seroconversion as the main cause of the association between BCP mutations and disease progression (15). Taking together the lines of evidence, it could be suggested that the prevalence of BCP mutations in each population might serve as a risk calculator and predictor for cirrhosis development in HBV carriers (16). Thus, patients with BCP mutant should consider prevention treatment for cirrhosis. The findings of this work showed that the prevalence of BCP mutations among the HBsAg positive patients in the south of Iran was relatively low (8.24%). However, Chu et al. ascertained that core promoter variants were found in 44% of patients with chronic HBV infection in the United States. The authors suggested that the influx of immigrants from East and Southeast Asia in recent years may cause this epidemiology of HBV infection in the United States (17). The considerable differences between these investigations and various populations may be due to several factors, including age, HBV genotype, ethnicity, previous antiviral therapy, and hepatic decompensation. Based on the reported observations, the mutations are more prevalent in genotypes B and C of HBV genotypes, which are the major genotypes in East and Southeast Asia and Australia (18), where more than half of the estimated carriers in the world live (19). These arguments are in agreement with the study of Chu et al. (17). On the other hand, genotype D is the major genotype of HBV in Iran, which has a lesser ability for BCP mutations. Therefore, it is reasonable that the prevalence of the mutation in core region of HBV in Iranian carriers are low (20, 21). Clinical manifestations of HBV infection differ from simple mental disorders (22-24) to life-threatening outcomes, depending on features of the infected person and genetic characteristics of the virus (25, 26). Several studies in recent years have proposed that HBV carriers with T1762/A1764 basal core promoter mutant are at increased risk for chronic hepatitis and hepatocellular carcinoma (27), while our results indicated a significant association between basal core promoter mutations and acute phase of hepatitis. In fact, this study demonstrated equal amounts of core promoter mutation in both of the acute and chronic phases of hepatitis. This observation is in agreement with the findings of two studies conducted by X Ren et al. and R Gérolami et al. The authors believed that the changes in the basal core promoter region of the hepatitis B virus DNA can increase the risk of acute and even the fulminant hepatitis (28, 29). However, the findings of this investigation should be presented with caution, as the present study was limited in several ways: first, the relatively small size of study population due to limited numbers of the visitors; second, lack of consideration of different HBV genotypes due to their genomic differences; finally, lack of consideration the racial differences and migrants.

#### 5. Conclusions

In conclusion, the prevalence of BCP variants was low in the south of Iran, and this mutation can lead to acute and chronic phase of viral hepatitis with the same chance. Therefore, healthcare authorities and blood transfusion centers can perform additional screening tests for BCP mutations, at least for high-risk groups, including old age, children, and immunocompromised patients. In future studies, it is suggested to consider different HBV genotypes and also the racial differences and migrants in larger projects.

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#### Conflict of Interest:

There is no conflict of interest to be declared.

#### Authors' contributions:

All authors contributed to this project and article equally. All authors read and approved the final manuscript.

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