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Original paper

Precore/core mutations of hepatitis B virus genotype D arising in different states of infection

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Abstract

Aim of the study: Precore/core variations and liver disease progression have been suggested. In this study, we aimed to determine the frequency of precore/core mutations in hepatitis B virus (HBV)-infected patients at various clinical stages.

Material and methods: In total, 73 HBV-infected patients including 26 inactive carriers (IC), 20 chronic active (CA), and 27 patients with liver cirrhosis/hepatocellular carcinoma (C/HCC) were randomly selected. The HBV DNA was extracted from the sera and subjected to nested PCR for amplification of precore/core region. The PCR product was then sequenced by the Sanger method.

Results: The stop codon of W28*(G1896A) was determined as the most prevalent mutation (55%) of the precore region. The comparison of groups also demonstrated that core substitutions at residues of S21, E40 and I105 (< 0.05) correlated with the development of the inactive carrier state. Furthermore, the total substitutions in Th epitopes (117-131) were significantly higher in the C/HCC group than the IC and CA groups (p = 0.001).

Conclusions: Our results indicated a high frequency of W28* mutation in HBV studied patients. Moreover, variations including S21, E40 and I105 and R151 that were mapped onto cellular epitopes might be related to inactive state development.

Key words: hepatitis B virus, precore, core, variation.

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Introduction

Nowadays, chronic hepatitis B infection remains a major cause of liver-related morbidity and mortality, even in the presence of an effective vaccine. It was predicted that 257 million people were living with chronic hepatitis B infection in 2015 [1]. A meta-analysis study in 2017 estimated that the prevalence rates of HBsAg and anti-HBcAb in Iran's general population were 1.79% (1.67% to 2.32%) and 13.59% (12.92% to 14.29%), respectively [2]. Moreover, the pooled prevalence of HBsAg among people who use drugs through injecting and otherwise was 4.8% and 2.9%, respectively [3]. Additionally, the pooled prevalence of HBsAg among Iranian hemodialysis patients was 4% [4].

The genome of HBV contains four overlapping open reading frames (ORFs) encoding the surface antigens (pre-s1, pre-s2 and s), precure (PC)/core (C) antigens, the polymerase, and a regulatory protein named X protein [5]. Based on the complete genome sequence, HBV strains are categorized into 10 genotypes, and it has been elucidated that some of them are more prone to mutations that contribute to the progression and severity of liver disease as well as poor antiviral responses, fibrosis, and hepatocellular carcinoma (HCC) progression [6]. Studies have shown that there is a relationship between variations in specific regions of the viral genome and progression to HBV-mediated liver diseases [6-8]. Previously, we described some mutations in the surface antigen sequence associated with the HBV infection outcome [9, 10].

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The core or capsid protein (HBcAg) and HBeAg are encoded on the precore/core ORF. Some variations in this frame which result in the conversion of the expression level, protein structure, encapsidation properties, and antigenicity could be associated with disease/infection stage [11-13]. It has also been indicated that HBeAg-negative chronic patients respond much less than HBeAg-positive cases to conventional interferon α therapy [14-16]. The HBeAg^{-}phenotype arises due to some substitutions and more particularly a stop codon W28* in the precore sequence which is associated with abrogation of expression in the HBeAg level [17, 18]. The studies on precore variations and the molecular mechanisms underlying the disease progression supported the role of HBeAg^{-} status in HCC development [5, 18].

In addition, some studies showed the importance of certain mutations in HBcAg, a protein with 183 residues length, in determining the clinical outcome [5]. HBcAg is an immunogenic protein, the most potent inducer of the immune response by cytotoxic T lymphocytes [19, 20]. Therefore, mutations in this region permit the virus to evade the host immune responses and establish a persistent infection [21-23]. Numerous HBcAg substitutions are associated with immune escape of the virus as well as more severe liver disease [5]. Moreover, Hayashi *et al.* showed that the core mutations such as T1938C (V13A) and A2051C (N51H) were correlated with HBV-related HCC and played a role in progression of liver disease in an Alaskan native population with HBV genotype F1b [24]. Additionally, they reported that A2051C increased the viral replication *in vivo* and *in vitro* [24]. In a study conducted in the Korean population, the five HBcAg mutations P5H/L/T, E83D, I97F/L, L100I, and Q182K/Stop were significantly more frequent in subjects with chronic hepatitis and cirrhosis [25]. Due to the host immune pressure in the course of viral persistence, accumulation of HBcAg mutations could occur and lead to the expansion of mutants that escape recognition by host immunity [5]. Determination of precore/core gene mutations in the immune epitopes at various clinical stages of HBV-infected patients would give additional insight into the role of these mutations in viral persistence and progression of liver damage. Therefore, in this study we aimed to determine the frequency of precore/core mutations in a small group of Iranian HBV-infected patients at various clinical stages and to clarify the association between precore/core mutations and disease progression.

Material and methods

Patient selection

In this cross-sectional study, 73 subjects including those with inactive carrier (IC) state, chronic active (CA) and cirrhosis/HCC (C/HCC) were enrolled consecutively from the Gastroenterohepatology Research Center, at Nemazee Hospital and Organ Transplantation Research Center at Central Abu-Ali Sina Hospital affiliated to Shiraz University of Medical Sciences during the years 2013 to 2017. All patients enrolled in the study were HBsAg positive; they were categorized into the three above-mentioned groups by a liver specialist according to biochemical, virological, and clinical records based on EASL guidelines [26]. The IC group included chronic patients with HBeAg negativity, a normal alanine aminotransferase (ALT)/aspartate aminotransferase (AST) level and < 2000 IU/ml viral load. The chronic active group consisted of chronic patients who were positive/negative for HBeAg, with an elevated level of ALT/AST and a viral load of > 2000 IU/ml up to 10^9 IU/ml. Cirrhosis and hepatocellular carcinoma patients were enrolled based on ultrasound scanning, histology grading, abnormal liver function tests, and α-fetoprotein levels. In prior studies we determined the HBV subgenotype of patients, and all patients were subgenotype D [9, 10].

Upon the patients' agreement, 5 ml of venous blood without anti-coagulation was taken from them. The sera samples were isolated and kept at –20°C until the experiment time. The study was approved by the local ethics committee of Shiraz University of Medical Sciences (IR.SUMS.Rec.1396.S530).

Viral DNA extraction and core gene amplification

The HBV genome was extracted from the patients' sera using the Cinnagen viral DNA extraction kit (Cin-

Table 1. Sequences of primers used in the nested PCR

Name	Sequence	Position	Product size
Forward HBC-1 Reverse HBC-1	5'-AATGTCAACGACCGACCTT-3' 5'-GADGGWGTTTTCCAATGAGG-3'	1679-2539nt	861 bp
Forward HBC-2 Reverse HBC-2	5'-GTAYTAGGAGGCTGTAGGCA-3' 5'-CCCACCTTATGAGTCCAAG-3'	1770-2476nt	707 bp

nagen Inc. Tehran, Iran), according to the manufacturer's instructions. After extraction, a nested PCR was performed using outer and inner primers specific for the precore/core region. Primers were designed based on the genomic sequences of B, C and D genotypes of HBV (Table 1).

The first round of PCR reaction contained 0.5 pmol of the outer primer pair, 5 μl of the extracted DNA, 1.5 mM of $MgCl_2$, 1 U of Taq DNA polymerase (Cinnagen Inc, Tehran, Iran), and 200 mM of each dNTP. The final volume of the PCR reaction was 25 μl. The first round of nested PCR was as follows: denaturation at 95°C for 5 min, followed by 30 cycles of 95°C for 40 s, 54°C for 45, 72°C for 50 s, and a final extension cycle performed at 72°C for 3 min.

In the nested round, 2 μl of the first round PCR amplified products was then subjected to reaction containing each of the inner primers. The second round nested PCR was performed as the first round, except with 35 cycles and annealing temperature of 56°C. Negative and positive controls were also used to evaluate the test validity.

Sequencing and multiple sequence alignment

PCR products were purified from the gel using PCR Product Purification Kit (MN Inc., Germany) and then sequenced by Sanger bidirectional sequencing using the internal primers. They were deposited in the NCBI (National Center for Biotechnology Information) data bank under accession numbers MG491124.1-MG491194.1. A group of precore/core reference/reliable sequences were obtained from the NCBI data bank regarding 7 main HBV genotypes and different genotype D sub-genotypes. The data resulting from sequencing were aligned with reference genomic sequence of HBV genotypes A-H using MEGA X software [Molecular Evolutionary Genetics Analysis across computing platforms (https://www.megasoftware.net/web_help_10/Citing_MEGA_In_Publications.htm)] to detect the putative amino acid substitutions. All differences between the patients' sequences and reference genomic sequence of genotype D were considered as variations.

Statistical analysis

For statistical analysis, SPSS software was employed. The χ^2 test was used for data analysis, and $p < 0.05$ was considered as significant. The results are presented as mean and standard deviation (SD).

Results

Patients' data

Out of 73 HBV patients, 27 were IC, 20 were CAH, and 26 were C/HCC. The mean age of the subjects in the IC, CA and C/HCC groups was 49.2 ±12.9, 40.7 ±14.2, and 50.9 ±11.8, respectively; the difference among the three groups was not significant ($p = 0.72$). Demographic characteristics and clinical data of the studied groups are shown in Table 2.

Variations of the precore sequence

Analysis and comparison among the retrieved sequences and reference ones revealed some types of substitutions in the precore region. The frequency distribution of mutations in hepatocellular carcinoma/cirrhosis, chronic active and inactive carrier groups was 26, 16 and 25, respectively. These

Table 2. Demographic and clinical data of the study groups –

Variable	Cirrhotic/HCC	Chronic active	Inactive carrier	*P*-value
No. of patients	26	20	27	–
Gender, *n* (%)				
Male	23 (88.5)	15 (75)	17 (63)	0.72
Female	3 (11.5)	5 (25)	10 (37)	
Mean age ±SD	50.9 ±11.8	40.7 ±14.2	49.2 ±12.9	0.094
ALT ±SD	48.8 ±27.7	40.3±24	21.5 ±9.6	< 0.001
AST ±SD	76.0 ±50.2	34±12.3	21.9 ±4.7	< 0.001
HBeAg positive, *n* (%)	3/26 (11.5)	7/20 (35)	0/27 (0)	< 0.01
HBeAb, *n* (%)	23/26 (88.46)	13/20 (65)	27/27 (100)	0.62
HBsAg positive, *n* (%)	26/26 (100)	20/20 (100)	27/27 (100)	–

ALT – alanine aminotransferase, AST – aspartate aminotransferase, SD – standard deviation

Table 3. Frequency of precore/core variations in the three studied groups

Residue	Hepatocellular carcinoma/cirrhosis (*n* = 26)	Chronic active (*n* = 20)	Inactive carrier (*n* = 27)	*P*-value
27	V/L(1)	–	V/I(1)	0.68
28 (Precore)	W28*(13)	W28*(9)	W28*(18)	0.693
29 (Precore)	G/D(12)	G/D(7)	G/D(6)	0.19
Core mutations				
12	T/S(6)	T/S(6)	T/S(10)	0.718
21	S/P(1), S/T(2)	S/G(1)	S/T(5), S/A(8), S/H(1)	0.006
25	P/T(9)	P/T(5)	P/T(4)	0.427
38	Y/C/L Y/F(2)	Y/F(2) Y/D(1)	Y/F(2)	0.682
40	E/D(2)	E/D(6)	E/D(9), E/Q(1)	0.04
45	P/S (3)	P/S(1)	P/S(5)	0.46
49	S/A(1)	S/T(1)	S/T(6)	0.11
59	I/T(3), I/Y(1), I/V(1)	I/F(1)	I/T(2), I/V(1), I/C(1)	0.458
64	E/D(7)	E/D(7), E/N(1)	E/D (4)	0.33
66	M/I(2), M/L(3)	M/L(1), M/K(1), M/I(1)	M/I(2)	0.757
67	T/N(3), T/S(1)	T/N(4)	T/N(4), T/S(3)	0.742
69	A/S(1), A/P(1)	A/G(2), A/S(2)	A/S(2)	0.413
77	E/Q(3), E/D(1)	E/D(1)	E/D(3), E/Q(1)	0.571
79	P/T(1), P/Q(1)	P/Q(3)	P/Q(5)	0.593
80	I/T(8), I/T, I/V(5), I/A(3)	I/T(8), I/V(3), I/A(3)	I/T(12), I/V(2), I/P(2), I/M(1), I/A(1)	0.95
87	S/T(3)	S/G(1)	S/G(3), S/H(1), S/R(1)	0.46
92	N/H(3)	N/H(1), N/T(1)	N/H(3), N/T(1)	0.9
93	M/L(2), M/W(1)	M/L(1), M/I(1)	M/V(1)	0.597
105	I/L(1)	–	I/L, I/V(4), I/T(2)	0.04
113	E/D(4)	E/Q(1), E/D(3)	E/D(6), E/Q(2)	0.596
116	I/L(5), I/V(4), I/K(1)	I/V(2)	I/L(2), I/V(2)	0.13
135	P/Q(3)	P/Q(1)	P/Q(1), P/T(1)	0.75
147	T/A(3), T/N(1), T/C(1)	T/C(1), T/A(1), T/S(1)	T/A(2), T/C(1)	0.777
149	V/I(4)	V/I(6)	V/I(10)	0.388
151	–	R/Q(1), R/S(1), R/P(1)	R/Q(6), R/G(1)	0.05
153	G/C(3)	G/C(1)	G/S(1)	0.543
155	S/T(4)	S/F (1), S/A (1), S/T (1)	S/T(7)	0.658
174	–	R/K(3)	R/K(2), R/T(1), R/I(2)	0.116
181	S/P(2), S/R(1)	S/P(1)	S/P(2)	0.75
182	Q/K(2), Q/*	Q/H(1)	Q/H(3)	0.752

mutations appeared more commonly in 27, 28 and 29 residues, as listed in Table 3. The stop codon of W28* was detected in 55% (40/73) of the patients, which was the most frequent change in the precore sequence, but the difference among the groups was not significant ($p = 0.69$). The G29D substitution detected in 32% (25/73) of the subjects was the second one, but its frequency among the studied groups was also not significant ($p = 0.19$).

Overall variations of core sequence among groups

Totally, the count of substitutions within the immune epitopes in the IC, CA and C/HCC groups was 200, 108, and 170 events, which was higher in IC subjects than CA and C/HCC ones ($p > 0.05$). The variations in these positions account for the virus immune

evasion. Additionally, some residues including S21 (24.6%), P25 (24.6%), E40 (24.6%), E64 (26%), T67 (20%), 80I (67.12%), E113 (21.9%), I116 (21.9%), V149 (27.3%), and S155 (19.1%) were determined as more variable positions alongside the core sequence of all patients (Table 3), and substitutions at S21 ($p = 0.006$), E40 ($p = 0.04$) and I105 ($p = 0.04$) were significantly higher in the IC subjects. No deletion/insertion event was found in the sequences when compared with reference ones, but rare stop codon mutations such as L101* and L76* were also detected.

Mutations in the cytotoxic T lymphocyte and T helper lymphocyte response regions

Several variations in immune recognition sites along the HBcAg protein were detected. The CTL epitopes that had been considered in this study included 18-27, 88-96, 130-140, and 140-151, whereas Th-epitopes were 1-20, 50-69, 81-105, 117-131, and 141-165. The core sequences analysis showed that 156, 86 and 122 substitutions occurred in the IC, CA, and C/HCC groups, respectively. The total number of CTL epitope mutations was 54, 29 and 40 in the IC, CA, and C/HCC groups, respectively. The rate of variation at CTL-related residue 151 was higher in the IC than the CA and C/HCC groups. The most dominant variations were seen in CTL epitopes located at site 130-151; however, all variations in epitopes were higher in the IC group than CA, but they were not statistically significant (Table 4).

In the case of T-helper epitopes, variation in positions E64, T67, I105, and S155 was more common among all the groups; however, only I105 was significantly different among them ($p = 0.04$). The substitution rates of epitopes including sites 50-69 and 141-165 were more frequent than other Th epitopes in all groups. In sum, the total substitutions in Th epitopes (117-131) were significantly higher in the C/HCC group than the IC and CA groups ($p < 0.001$).

Table 4. Number of amino acid substitutions alongside the entire core sequences

Hepatocellular carcinoma/cirrhosis (*n* = 26)	Chronic active (*n* = 20)	Inactive carrier (*n* = 27)	Cell subsets	Epitope sequence	*P*-value
S21T(2)/P, F24Y(2), P25T(9), V27L	S21G/T, P25T(5)	S21T(4)/A(8)/H, F24Y, P25T(4), S26A, V27I	CTL cell	18-27	0.25
N92H(3), M93L(2)/W	N92H/T, M93V(2)/I/L, G94A	T91S/E, N92H(3)/T, M93V	CTL cell	88-96	0.921
P130L/Q, A131P, Y132T(2), R133G, P135Q(3), I139L, L143P, T146S, T147A(3)/C, V149I(4)	P130I, A131G P135Q, L143P, T147A/C, V149I(6), R151S/Q/P	A131P, P135T/Q, L140*, T142M, L143P, T146S, T147A(2)/C, V149I(10), R151Q(6)/G	CTL cell	130-151	0.679
T12S(6), V13L, E14Q/D	T12S(6), E14Q	D2N, T12S(10) E14Q(3), L16I(4)	Th1 cell	1-20	0.297
P50A, A54V, R56G I59Y/T(3)/V, C61R, W62C, G63W, E64D(7), M66I/L, T67N(3)/S, A69S/P	L55I(2), I59F, C61W W62G, E64D(7)/N, M66I/K/L, T67N(4) L68P, A69G(2)/S(2)	P50A(3), L55I Q57L/R, I59T(2)/V/C C61R, G63A(2), E64D(4), M66I(2), Y67S(3)/N(4), L68P, A69S(2)	Th1 cell	50-69	0.857
L84Q, S87T(3), N92H(3) M93L(2)/W, F97V, R98Q, L100R, W102G, H104Y, I105L	S87G, N92H/T, M93L/I, G94A	L84P/Q, S87G(3)/H/R, T91S/E N92H(3), M93V L101*, I105L/V(4)/T(2)	Th1 cell	81-105	0.25
E117G, Y118F, L119W, V120A, F122S, V124G, W125G(2), R127A/G, T128L, P129L, P130L/Q, A131P	P130I, A131G	A131P	Th1 cell	117-131	0.001
L143P, T147A(3)/N/C, V149I(4), G153C(3) S155T(4), R157E, R165K	L143P, T147S/C/A V149I(6), R151P/S/Q G153C, S155F/T/A	T142M, L143P T146S, T147A(2)/C V149I(10), R151Q(6)/G, G153S, S155T(7), P156T(2)	Th1 cell	141-165	0.329
W71G, V72W, G73V L76V, E77Q(3)/D D78A, P79Q/T I80T(9)/V(5)/A(3) L84Q, S87T(3)	W71R, G74V E77D, P79Q(3) I80T(8)/V(3)/A(3) S87G	W71R, G74S(3)/A/V L76*, E77D(3)/Q P79Q(5), I80T(12)/V(2)/A(1)/P(2)/M, L81I, L84P/Q, S87G(2)/H/R	B cell	71-89	0.512
R127A/G, T128L, P129L, P130L/Q, A131P, Y132T, R133G, P135Q(3)	P130I, A131G, P135Q	A131P, P135Q/T	B cell	126-135	0.94

Mutations in the B lymphocyte epitope region

Sequence analysis of HBcAg indicated 108 mutations in the target epitopes for B-cells, as shown in Table 4. Among them, the most variable residues were located on epitope 71-89. The total frequency of B-cell epitope variations was determined to be 42, 24 and 42 for the IC, CA and C/HCC groups, respectively.

Discussion

Although the HBV vaccine was introduced more than 35 years ago, chronic HBV infection is one of the most important health problems worldwide today. Iran implemented general HBV vaccination in 1993 and is now classified as a low prevalence country with around 1,347,000 chronic HBV infections [2, 27].

The precore/core variations would possibly determine the fate of HBV infection as they modify encapsidation, HBeAg production, immune response, inflammation, ER , and DNA damage [18]. Some studies have reported that HBV infected patients with the presence of precore/core mutations significantly more often developed severe liver disease and HCC [18, 28].

The result of our study showed that a precore point mutation, G1896A, that converts the tryptophan to a stop codon (W28*), was the most common variation in the patients, as detected in chronic HBV in Asia and the Mediterranean region [18]. This mutation disrupted HBeAg production and was suggested to be involved in disease progression toward HCC [29]. The frequency of this mutation was higher in the C/HCC group than the IC and CA groups, but it was not statistically significant. In the same line, in another study in Shiraz, Iran, Taghavi *et al.* reported that 31.8% (14/44) of patients had mutations in the precore region (G1896A) [30]. Moreover, Kim *et al.* reported no significant correlation between W28* and higher susceptibility to HCC [25]. In addition, it has been recently reported that chimeric mice expressing a recombinant virus containing the PCG1896A or basal core promoter (BCP)/PC/2051 mutations demonstrated higher levels of virus replication and viral protein expression than mice expressing the wild-type strain [24]. Also, microarray analysis of the liver transcriptome of these mice showed increased expression of the genes involved in cell proliferation and hepatocarcinogenesis in comparison with the control group [24].

Our results also showed G29D precore mutation at the highest frequency in the C/HCC group among the groups, but the difference was not statistically significant. In this regard, a meta-analysis study showed a significant correlation between G29D mutation and higher risk of HCC [31].

On the core protein, the types of substitutions including inside and outside of epitopes were suggested to be important. This investigation indicated that numerous substitutions in the immune epitopes were coincidently associated with more severe liver disease. The substitution E180A which was not mapped in an epitope region was suggested to be significantly associated with disease progression by other strategies [32]. There were some variations such as E77Q, E113Q, S181P/H and Q182K/*Stop outside of the epitope regions which have previously been reported to accumulate with disease progression [33-35]. However, in spite of their prevalence, our data showed that E80Q/D, E113D/Q, S181P/R and Q182K/*Stop variations were not statistically significantly different among the groups. Instead, as a new finding, the prevalence of E40D/Q was significantly higher in the IC subjects than CA and C/HCC groups. Further mechanisms accounting for these mutations in infection progression require additional molecular and virologic investigations.

The core substitutions of epitopes which are associated with severe liver disease have been reported by others. Jia *et al.* investigated the association between HBc mutations and the post-operative prognosis of HBV-related HCC, which demonstrated that the HBc E77 mutation was more associated with shorter overall survival than other mutations [5]. Al-Qahtani *et al.* reported 6 core mutations (F24Y, E64D, E77Q, A80I/T/V, L116I, and E180A) related to the progression of the disease to cirrhosis and HCC [32]. They also found that F24Y, E64D, and V91S/T mutations were located in the T-cell epitope regions, and E77Q, A80I/T/V, and L116I were located within the B-cell epitope regions [32]. The comparison of core sequence among our groups indicated that substitutions at residues S21, E40 and I105 were possibly accelerating the development of the IC state. This was also supported by amino acid changes at the CTL epitopes including S21, P25 and V149 that were significantly more frequent in the IC group. In line with this, Ghabeshi *et al.* reported that some mutations of HBc gene sequence in the T helper, CTL and B cell epitopes in asymptomatic HBV infected blood donors can cause a decrease in HBc and HBe antigenicity and an increase in escape mutants [36]. In addition, the rate of mutation at position 21 (S/T, S/A and S/H) in the CTL epitope in IC subjects was significantly higher than the CA and C/HCC groups. In theory, mutations in the core immune epitopes would subvert the immune responses to permit persistent HBV infection. It was also reported

that a distribution pattern of core mutations detected in the immune and non-immune regions was associated with progression of the disease [18].

The present study also demonstrated that the rates of F24Y, E64D, E77Q, L116I, and E180A mutations were higher in the C/HCC patients than the other groups; however, these differences were not statistically significant. Recently, Zhang *et al.* found that HBc L60V variation was associated with higher viral loads, necroinflammation of the liver, and probably a poor prognosis [37]. They proposed that this variation could influence both virus replication and T cell responses [37]. Mohamadkhani *et al.* suggested that the frequency of HBc mutations in the CTL epitope regions and C-terminal domain was associated with a higher stage of fibrosis [38]. Likewise, similar studies indicated that mutations of CTL epitopes at the C-terminal of the core might enhance the development of fibrosis [38]. In sum, there are several mutations considered as effective variations to enhance the progression of the liver disease. Accumulation of these mutations during viral persistence could be the result of escape recognition of the infected cell by the immune system and progression of liver impairment. Therefore, more efforts should be made to understand the effect of these mutations on the liver disease progression during HBV infection.

Some studies have shown that the locations of effective mutations are definitely mapped more on specific regions such as the 80-120 sequence [25, 39-41]. In our study, there was not a similar pattern over different parts of the core. Instead, it was found that the rate of substitutions in the IC group was higher than in the other groups.

Our study had some limitations such as a small sample size as well as a lack of available data regarding the viral replication parameters. However, these results help us to know the frequency of precore/core mutations in HBV infected patients in Fars province and greatly improve our understanding of precore/core mutations, mostly within the immune epitopes.

Conclusions

In conclusion, it suggested that variations including S21, E40, I105 and R151 which are located on cellular epitopes are associated with the immune inactive state in HBV-infected patients. Furthermore, more than half of the HBV-infected subjects harbored the stop codon W28* of the precore region that is responsible for the HBeAg negative state. However, a more detailed study on a larger population of HBV-infected patients is recommended to confirm this claim.

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Disclosure

The authors declare no conflict of interest.

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