The Analysis of Hepatitis B Virus Precore/core Mutations Arising in Different States of Infection

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Abstract

Background: In hepatitis B virus (HBV) infection, an association between Precore/Core variations and liver disease progression has been suggested. In this study, we aimed to determine the frequency of Precore/Core mutations in HBV-infected patients at various clinical stages.

Methods: Totally, 73 HBV-infected patients including 26 inactive carriers, 20 chronic active and 27 patients with liver cirrhosis/hepatocellular carcinoma were randomly selected. The HBV DNA was extracted from the sera and subjected to nested PCR for amplification of pre-core/core region. The PCR product was then sequenced by Sanger method.

Results: The stop codon of W/*28 was determined as the most prevalent mutation (55%) of pre-core region. The comparison of groups also demonstrated that core substitutions at residues of S21, E40 and I105 (<0.05) correlated with the development of inactive carrier state. Furthermore, the total substitutions in Th epitopes (117-131) were significantly higher in C/HCC group than IC and CA groups (P=0.001).

Conclusion: Our results indicated a high frequency of W/*28 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.

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).

Genome of HBV contains four overlapping open reading frames (ORFs) encoding the surface antigens (pre-s1, pre-s2 and s), pre-core (pc) /core (c) antigens, the polymerase, and a regulatory protein named X protein(2). Based on 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(3). Studies have shown that there is a relationship between the variation in specific regions of the viral genome and progression to HBV-mediated liver diseases (3–5). Previously, we described some mutations in the surface antigens sequence associated with HBV infection outcome(6, 7).

The core or capsid protein (HBcAg) and HBeAg are encoded on 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 (8–10). It has also been indicated that HBeAg-negative chronic patients respond much less than HBeAg-positive cases to conventional interferon-alpha therapy (11–13). The $HBeAg^{-}$ phenotype arises due to some substitutions and more particularly a stop codon W28* in pre-core sequence which is associated with a significant decrease in

the HBeAg level (14, 15). The studies on pre-core variations and the molecular mechanisms underlying the disease progression advocate the role of $HBeAg^-$ status in HCC development (2, 15).

In addition, some studies showed the importance of certain mutations in HBcAg, a protein with 183 residues length, in determining the clinical outcome(2). In one side, HBcAg is an immunogenic protein, the most potent inducer of immune response by cytotoxic T lymphocyte (16, 17). Therefore, mutations in this region permit the virus to evade from the host immune responses and establish a persistent infection (18–20). Numerous HBcAg substitutions are associated with immune escaping of the virus as well as more severe liver disease (2). Moreover, Hayashi et al. showed that the core mutations such as T1938C (V13A) and A2051C (N51H) are correlated with HBV-related HCC and play a role in progression of liver disease in Alaskan native population with HBV genotype F1b(21). Additionally, they reported that A2051C increased the viral replication in vivo and in vitro(21). In a study conducted in the Korean population, five HBcAg mutations P5H/L/T, E83D, I97F/L, L100I, and Q182K/Stop were significantly higher in subjects with chronic hepatitis and cirrhosis (22). Due to 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(2). 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.

Materials And Methods

Patient's selection

In this cross-sectional study, 73 subjects including those with inactive carriers (IC) state, chronic active hepatitis (CAH), 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 a period from 2013 to 2017. Patients were categorized into three above-mentioned groups by a liver specialist regarding the diagnosis by biochemical, virological and clinical records. Clinical and demographic information was collected using their medical records. 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.

Viral DNA Extraction and Core gene Amplification

The HBV genome was extracted from the patient's sera by *Cinnagen* viral DNA extraction kit (Cinnagen Inc. Tehran, Iran) regarding recommended instruction. After extraction, a nested PCR was performed

using outer and inner primers specific for precore/core region. Primers were designed based on the genomic sequences of B, C and D genotypes of HBV (Table 1).

Table 1
The sequences of primers used in the Nested PCR

Name	Sequence	Product size
Forward HBC- 1	5′-AATGTCAACGACCGACCTT-3′	861 bp
Reverse HBC- 1	5′-GADGGWGTTTTCCAATGAGG-3′	
Forward HBC – 2	5′-GTAYTAGGAGGCTGTAGGCA-3′	707 bp
Reverse HBC- 2	5′-CCCACCTTATGAGTCCAAG-3′	

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

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 in 35 cycle and annealing temperature of 56 °C. Negative and positive controls were also used to evaluate the test validity.

Sequencing and multiple sequences 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 NCBI (National Center for Biotechnology Information) data bank under accession numbers: MG491124.1-MG491194.1. A group of precore/core reference/reliable sequences were adopted from 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 MEGA7 software to detect the putative amino acid substitutions. All differences between the patient's sequences and reference genomic sequence of genotype D were considered as variations.

Statistical Analysis

For statistical analysis, SPSS software was employed. Chi square test was used for data analysis and $P < 0.05$ was considered as significant.

Results

Patient's 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 was not significant among the three groups (P = 0.72). Demographic characteristics and clinical data of the studied groups are shown in Table 2.

Table 2
Demographic and clinical data of the study groups

		Cirrhotic/HCC	Chronic active	Inactive carrier	P- value
No. of patients		26	20	27	
Gender	**Male**	23 (88.5%)	15(75%)	17 (63%)	P = 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	P = 0.094
ALT*±SD		48.8 ± 27.7	40.3 ± 24	21.5 ± 9.6	P < 0.001
AST*±SD		76.0 ± 50.2	34 ± 12.3	21.9 ± 4.7	P < 0.001

*; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase ;ALP: Alkaline phosphatase

The variations of pre-core sequence

Analysis and comparison among the retrieved sequences and reference ones revealed some types of substitutions in the pre-core region. These mutations appeared more commonly in V27, W28 and G29D residues, as listed in Table 3. The stop codon of W/*28 was detected in 55% (40/73) of the patients that was the most frequent change in the pre-core sequence, but the difference was not significant among the groups (P = 0.69). The G29D substitution detected in 32% (25/73) of the subjects was the second one, but its frequency was not also significant among the studied groups (P = 0.19).

Table 3
The frequency of Precore/Core variations in the three studied groups

Position	Hepatocellular carcinoma (N = 26)	Chronic active (N = 20)	inactive carrier (N = 27)	P-Value
27	V/L(1)	-	V/I((1)	0.68
28 (Pre-core)	W/*28 (13)	W/*28 (9)	W/*28 (18)	0.693
29 (Pre-core)	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**

Position	Hepatocellular carcinoma (N = 26)	Chronic active (N = 20)	inactive carrier (N = 27)	P-Value
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

The overall variations of core sequence among groups

Totally, the frequency 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 on this 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 events were probed 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 herein. 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 CA and C/HCC groups. The most

dominant variations were seen in CTL epitopes located on 130–151 site; however, all variations in epitopes were higher in the IC group than CA but not statistically significant (Table 4).

Table 4
The number of amino acid substitutions alongside the entire core sequences

Hepatocellular carcinoma (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**

Hepatocellular carcinoma (N = 26)	Chronic active (N = 20)	Inactive carrier (N = 27)	Cell subsets	Epitope Sequence	*P value*
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

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 substitutions rate of epitopes including 50–69 and 141–165 sites were more frequent than other Th epitopes in all groups. In sum, the total substitutions in Th epitopes (117–131) were significantly higher in C/HCC group than IC and CA groups ($P < 0.001$).

Mutations in the B lymphocyte epitopes 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 number of B-cell epitope variations was determined to be 42, 24 and 42 for the IC, CA and C/HCC groups, respectively. The results showed that the frequency of substitutions was higher in the IC and C/HCC groups than the CA group but not statistically significant.

Discussion

The variations of pre-core/core would possibly determine the fate of HBV infection as they modify encapsidation, HBeAg production, immune response, inflammation, ER stress and DNA damage(15). Some studies reported that HBV infected patients with the presence of precore/core mutations were significantly developed to severe liver disease and HCC(15, 23).

The result of our study showed that a point mutation of pre-core, G1896A that convert the tryptophan to a stop codon (W/*28) was the most common variation in the patients, as detected in chronic HBV in Asia and the Mediterranean region (15). This mutation destroyed the HBeAg production and was suggested to be effective on virus replication and somehow disease progression toward HCC(24). The number of this mutation was higher in the C/HCC group than the IC and CA groups but not significantly. In the same line that, in another study in Shiraz, Iran, Taghavi et al. has reported 31.8% (14/44) of patients had mutations in the precore region (G1896A)(25). Moreover, Kim et al. reported no significant correlation between W/*28 and higher susceptibility to HCC(22). In addition, recently it has been reported that chimeric mice expressing recombinant virus containing the PC G1896A or BCP/PC/2051 mutations demonstrated higher levels of virus replication and viral protein expression than the mice expressing wild-type strain(21). 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(21).

Our result also showed G29D precore mutation at the highest frequency in the C/HCC group among the groups, but the differenc ewas not statistically significant. In this regard, a meta-analysis study showed a significant correlation between G29D mutation and higher risk of HCC(26).

On the core protein, types of substitutions including inside and outside of epitopes were suggested to be impressive. This investigation indicated that numerous substitutions in the immune epitopes were coincidently associated with more severe liver disease. The substitution such as E180A which was not mapped in an epitope region was suggested to be significantly associated with disease progression by another strategies (27). 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 by the disease progression (28–30). However, in spite of their prevalence, our data showed that E80Q/D, E113D/Q, S181P/R and Q182K/*Stop variations were not statistically significant 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 Ja 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(2). Al-Qahtani et al. reported 6 core mutations (F24Y, E64D, E77Q, A80I/T/V, L116I, and E180A) related with the progression of the disease to cirrhosis and HCC(27). They also showed 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(27). The comparison of core sequence among our groups indicated that substitutions at residues S21, E40 and I105 were possibly accelerating the development of 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, Soad Ghabeshi et al. reported that some mutation 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 increase in escape mutants(31). The same mutations were found in our study in the amino acids at positions 21 (T/A to S), 25 (A/Q/T to P) in the CTL epitopes ,40 (D/P to E), 64 (D to E) in the T helper epitopes and 79 (N/R to P), 80 (T to I),109 (A/ I /M to T),113 (P/Q to E), 114 (I/N to T) in the B cell epitopes although those substitutions were different from ours in some positions. In addition, the rate of mutation at the position 21 (S/T, S/A and S/H) in the CTL epitope in IC subjects was significantly more prevalent 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 the non-accidental distribution of core mutations detected in the immune and non-immune regions was associated with progress of the disease(15).

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 mutations were not statically significant. Recently, Yu Zhang et al. found that HBc L60V variation was associated with higher viral loads, necroinflammation of the liver and it may be related with a poor prognosis(32). They proposed that this variation could influence both virus replication and T cell responses(32). Mohamadkhani et al. suggested that the frequency of HBc mutations in the CTL epitope regions and C-terminal domain is associated with higher stage of fibrosis(33). Likewise, similar studies indicated that mutations of CTL epitopes at C-terminal of the core might enhance the development of fibrosis (33). In sum, there are several mutations considered as effective variations to enhance the progress 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 these mutations' effect 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 80–120 sequence (22, 34–36). 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 determined to be higher than the other groups.

Our study had some limitations such as small sample size, no available data regarding the viral replication parameters and the moiety of sampling that was cross-sectional. However, these results help us to know the frequency of precore/core mutations in HBV infected patient in Fars province and greatly improves our understanding of precore/core mutations, mostly within the immune epitopes.

Conclusions

In conclusion, it could be suggested that variations including S21, E40, I105 and R151 which are located on cellular epitopes are associated with the immune inactive state, due to HBV immune escape. Furthermore, more than half of the HBV infected subjects harbor stop codon of W/*28 of pre-core region that is responsible for HBeAg negative state. However, a more detailed study on a larger population of HBV infected patients is recommended to confirm this claim.

Abbreviations

HBV: Hepatitis B Virus, ORFs: Open Reading Frames, pc: pre-core, c: core, HCC: Hepatocellular Carcinoma, IC: inactive carriers, CAH: Chronic Active Hepatitis, NCBI: National Center for Biotechnology Information

Declarations

Ethics approval and consent to participate

The study was approved by the local ethics committee of Shiraz University of Medical Sciences.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

All the authors declared that there is no conflict of interest.

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Authors' contributions

The aim of this study is to investigate the effect of Hepatitis B virus Precore/core mutations on the HBV infection outcome. Concept and design: Sarvari J and Hosseini SY; patients' selection: Moini M and Malekhosseini SMA; Bench work: Sanaei N, Zahra Salehi Dehno SZ, Mahmoudi Asl M; Manuscript drafting and data analysis: Seyed Mohammad Ali Hashemi; Critical revision of manuscript: Sarvari J, Hossini, Moini M and Malekhosseini SMA. The manuscript has been read and approved by all named authors.

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References

1. WHO. Hepatitis B. https://wwwwhoint/news-room/fact-sheets/detail/hepatitis-b.

2. Jia Ja, Li H, Wang H, Chen S, Wang M, Feng H, et al. Hepatitis B virus core antigen mutations predict post-operative prognosis of patients with primary hepatocellular carcinoma. The Journal of general virology. 2017;98(6):1399.
3. Paudel D, Suvedi S. Hepatitis B Genotyping and Clinical Implication. Hepatitis B and C: IntechOpen; 2019.
4. Sugiyama M, Tanaka Y, Kurbanov F, Maruyama I, Shimada T, Takahashi S, et al. Direct cytopathic effects of particular hepatitis B virus genotypes in severe combined immunodeficiency transgenic with urokinase-type plasminogen activator mouse with human hepatocytes. Gastroenterology. 2009;136(2):652-62. e3.
5. Sugiyama M, Tanaka Y, Kato T, Orito E, Ito K, Acharya SK, et al. Influence of hepatitis B virus genotypes on the intra-and extracellular expression of viral DNA and antigens. Hepatology. 2006;44(4):915-24.
6. Hosseini SY, Sanaei N, Fattahi M-R, Malek-Hosseini SA, Sarvari J. Association of HBsAg mutation patterns with hepatitis B infection outcome: Asymptomatic carriers versus HCC/cirrhotic patients. Annals of hepatology. 2019.
7. Taghiabadi M, Hosseini SY, Gorzin AA, Taghavi SA, Monavari SHR, Sarvari J. Comparison of pre-S1/S2 variations of hepatitis B virus between asymptomatic carriers and cirrhotic/hepatocellular carcinoma-affected individuals. Clinical and experimental hepatology. 2019;5(2):161.
8. Ito K, Kim K-H, Lok AS-F, Tong S. Characterization of genotype-specific carboxyl-terminal cleavage sites of hepatitis B virus e antigen precursor and identification of furin as the candidate enzyme. Journal of virology. 2009;83(8):3507-17.
9. Baumeister MA, Medina-Selby A, Coit D, Nguyen S, George-Nascimento C, Gyenes A, et al. Hepatitis B virus e antigen specific epitopes and limitations of commercial anti-HBe immunoassays. Journal of medical virology. 2000;60(3):256-63.
10. Laine S, Thouard A, Derancourt J, Kress M, Sitterlin D, Rossignol J-M. In vitro and in vivo interactions between the hepatitis B virus protein P22 and the cellular protein gC1qR. Journal of virology. 2003;77(23):12875-80.
11. Saikia N, Talukdar R, Mazumder S, Khanna S, Tandon R. Management of patients with HBeAg-negative chronic hepatitis B. Postgraduate medical journal. 2007;83(975):32-9.
12. Hui C-K, Lau GK. Treatment of hepatitis B e antigen-negative patients. Current treatment options in gastroenterology. 2007;10(6):474-82.
13. Yeh M-L, Peng C-Y, Dai C-Y, Lai H-C, Huang C-F, Hsieh M-Y, et al. Pegylated-interferon alpha therapy for treatment-experienced chronic hepatitis B patients. PloS one. 2015;10(4):e0122259.
14. Tong S, Kim K-H, Chante C, Wands J, Li J. Hepatitis B virus e antigen variants. International journal of medical sciences. 2005;2(1):2.
15. Kim H, Lee S-A, Do SY, Kim B-J. Precore/core region mutations of hepatitis B virus related to clinical severity. World journal of gastroenterology. 2016;22(17):4287.

16. Shanmugam S, Velu V, Nandakumar S, Madhavan V, Shanmugasundaram U, Shankar EM, et al. Low frequency of precore mutants in anti-hepatitis B e antigen positive subjects with chronic hepatitis B virus infection in Chennai, Southern India. J Microbiol Biotechnol. 2008;18(10):1722-8.
17. Kim H, Jee Y, Mun H, Song B, Park J, Hyun J, et al. Comparison of full genome sequences between two hepatitis B virus strains with or without preC mutation (A1896) from a single Korean hepatocellular carcinoma patient. Journal of microbiology and biotechnology. 2007;17(4):701.
18. Kim D, Lyoo KS, Smith D, Hur W, Hong SW, Sung PS, et al. Number of mutations within CTL-defined epitopes of the hepatitis B Virus (HBV) core region is associated with HBV disease progression. Journal of medical virology. 2011;83(12):2082-7.
19. Bock C-T, Buerke B, Tillmann HL, Tacke F, Kliem V, Manns MP, et al. Relevance of hepatitis B core gene deletions in patients after kidney transplantation. Gastroenterology. 2003;124(7):1809-20.
20. Preikschat P, Günther S, Reinhold S, Will H, Budde K, Neumayer HH, et al. Complex HBV populations with mutations in core promoter, C gene, and pre-S region are associated with development of cirrhosis in long-term renal transplant recipients. Hepatology. 2002;35(2):466-77.
21. Hayashi S, Khan A, Simons BC, Homan C, Matsui T, Ogawa K, et al. An association between core mutations in hepatitis B virus genotype F1b and hepatocellular carcinoma in Alaskan native people. Hepatology. 2019;69(1):19-33.
22. Kim D-W, Lee S-A, Hwang E-S, Kook Y-H, Kim B-J. Naturally occurring precore/core region mutations of hepatitis B virus genotype C related to hepatocellular carcinoma. PloS one. 2012;7(10):e47372.
23. Alexopoulou A. Mutants in the precore, core promoter, and core regions of Hepatitis B virus, and their clinical relevance. Annals of Gastroenterology. 2009:13-23.
24. Laskus T, Persing DH, Nowicki MJ, Mosley JW, Rakela J. Nucleotide sequence analysis of the precore region in patients with fulminant hepatitis B in the United States. Gastroenterology. 1993;105(4):1173-8.
25. Taghavi SA, Tabibi M, Eshraghian A, Keyvani H, Eshraghian H. Prevalence and clinical significance of hepatitis B Basal core promoter and precore gene mutations in southern Iranian patients. Hepatitis monthly. 2010;10(4):294.
26. Liao Y, Hu X, Chen J, Cai B, Tang J, Ying B, et al. Precore mutation of hepatitis B virus may contribute to hepatocellular carcinoma risk: evidence from an updated meta-analysis. PLoS One. 2012;7(6):e38394.
27. Al-Qahtani AA, Al-Anazi MR, Nazir N, Abdo AA, Sanai FM, Al-Hamoudi WK, et al. The correlation between hepatitis B virus precore/core mutations and the progression of severe liver disease. Frontiers in cellular and infection microbiology. 2018;8:355.
28. Zhang D, Ma S, Zhang X, Zhao H, Ding H, Zeng C. Prevalent HBV point mutations and mutation combinations at BCP/preC region and their association with liver disease progression. BMC infectious diseases. 2010;10(1):271.
29. Lee S-A, Kim K-J, Kim D-W, Kim B-J. Male-specific W4P/R mutation in the pre-S1 region of hepatitis B virus, increasing the risk of progression of liver diseases in chronic patients. Journal of clinical

microbiology. 2013;51(12):3928-36.
30. Carman WF, Thomas HC. Genetic variation in hepatitis B virus. Gastroenterology. 1992;102(2):711-9.
31. Hosseini phD SM, Sharifi phD Z. Molecular Evaluation of HBV Core Gene Mutations in Asymptomatic HBV Infected Blood Donors in Iran. Archives of Iranian medicine. 2014;17(11):759.
32. Zhang Y, Ren Y, Wu Y, Zhao B, Qiu L, Li X, et al. The L60V variation in hepatitis B virus core protein elicits new epitope-specific cytotoxic T lymphocytes and enhances viral replication. Journal of virology. 2013;87(14):8075-84.
33. Mohamadkhani A, Jazii FR, Poustchi H, Nouraein O, Abbasi S, Sotoudeh M, et al. The role of mutations in core protein of hepatitis B virus in liver fibrosis. Virology journal. 2009;6(1):209.
34. Sendi H, Mehrab-Mohseni M, Shahraz S, Norder H, Alavian S-M, Noorinayer B, et al. CTL escape mutations of core protein are more frequent in strains of HBeAg negative patients with low levels of HBV DNA. Journal of Clinical Virology. 2009;46(3):259-64.
35. Zhu Y, Jin Y, Cai X, Bai X, Chen M, Chen T, et al. Hepatitis B virus core protein variations differ in tumor and adjacent nontumor tissues from patients with hepatocellular carcinoma. Intervirology. 2012;55(1):29-35.
36. Mondal R, Khatun M, Ghosh S, Banerjee P, Datta S, Sarkar S, et al. Immune-driven adaptation of hepatitis B virus genotype D involves preferential alteration in B-cell epitopes and replicative attenuation—an insight from human immunodeficiency virus/hepatitis B virus coinfection. Clinical Microbiology and Infection. 2015;21(7):710. e11-. e20.