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).

| Name                  | Sequence                                           | Product size |
|-----------------------|----------------------------------------------------|--------------|
| Forward HBC-1         | 5’-AATGTCAGCGACCGACCTT-3’                          | 861 bp       |
| Reverse HBC-1         | 5’-GADGGWGTGTTCCAATGAGG-3’                         |              |
| Forward HBC-2         | 5’-GTAYTGGAGGCTGTAGGCA-3’                          | 707 bp       |
| Reverse HBC-2         | 5’-CCCACCTTATGACTTCAAG-3’                          |              |

The first round of PCR reaction contained 0.5 pmol of outer primer pair, 5 µL of the extracted DNA, 1.5 mM MgCl₂, 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 95°C 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**

| Position | Hepatocellular carcinoma (N = 26) | Chronic active (N = 20) | Inactive carrier (N = 27) | P-Value |
|----------|-----------------------------------|-------------------------|---------------------------|---------|
| 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)                    | 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)/A8/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 | 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)              | Th1 cell     | 1–20             | 0.297   |
| P50A, A54V, R56G                  | L55I(2), I59F, C61W      | P50A(3), L55I               | Th1 cell     | 50–69            | 0.857   |
| I59Y/T(3)/V, C61R, W62C, G63W, E64D(7), M66I/L, T67N(3)/S, A69S/P | W62G, E64D(7)/N, M66I/K/L, T67N(4), L68P, A69G(2)/S(2) | 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)            | S87G, N92H/T, M93L/I, G94A | L84P/Q, S87G(3)/H/R, T91S/E, N92H(3), M93V | Th1 cell | 81–105 | 0.25    |
| M93L(2)/W, F97V, R98Q, L100R, W102G, H104Y, I105L | | | | | |
| 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) | 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 |

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 difference was 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 statistically 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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