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REVIEW ARTICLE

Mutations within the HBc Gene of the Hepatitis B Virus: A Study on Iranian Patients

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SUMMARY

Background: **Hepatitis B virus (HBV) is a serious risk factor for several severe liver diseases such as cirrhosis and hepatocellular carcinoma. HBV, like other viruses, uses several mechanisms to escape from specific immune responses including the use of mutations in the genome which lead to epitope variations. There are several immune responses, including T helper cells, cytotoxic T lymphocytes, and B cells, against the core antigen of HBV (HBcAg) that can lead to HBV eradication. Therefore, mutations within the HBc gene can lead to escape from immune responses by HBV and, hence, understanding the prevalence of HBc mutations among a specific population can be helpful for future treatment and vaccination. This review addresses the recent information regarding the prevalence of mutations within the HBc gene among Iranian HBV infected patients.**
Methods: **The data presented here was collected gene sequences reported from Iran to the NCBI nucleotide Gen Bank.**
Results: **Results showed that the prevalence of HBc gene mutations is frequent in Iranian HBV infected patients.**
Conclusions: **Based on our searches it seems that escape from immune responses is a plausible reason for the high prevalence of HBc gene mutations among Iranian HBV infected patients.**
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KEY WORDS

hepatitis B infection, HBc gene, mutation

LIST OF ABBREVIATIONS

HBV - hepatitis B virus
HBcAg - hepatitis B core antigen
HBsAg - hepatitis B surface antigen
OBI - occult HBV infection
NCBI - National Center for Biotechnology Information
CTL - cytotoxic T lymphocyte
HLA - human leukocyte antigen
MHC - major histocompatibility complex
CD - cluster of differentiation

INTRODUCTION

Hepatitis B virus (HBV) is a main cause of human liver diseases [1-3]. Dysfunction and diseases of liver cells including cirrhosis and hepatocellular carcinoma are the most frequent clinical presentations of hepatitis B [4,5]. Recent studies revealed that many patients suffer from long term forms of HBV infection including chronic, asymptomatic, and occult (OBI) hepatitis B infection [6-9].

It appears that the quality and quantity of immune responses against HBV infection determines the fate of infection [9]. It has been shown that HBV may alter specific immune responses through a process of selec-

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tive advantage, meaning that an efficient and complete response to wild-type HBV may result in eradication from the host whereas a cell infected by HBV, harboring a specific mutation, may escape the host's immune system [10], hence the term 'escape mutation'. This could lead to a long term infection of cells by the mutant-containing HBV. The HBc gene encodes the hepatitis core antigen (HBcAg) which is the epitope that elicits several immune cell responses including those by T helper cells (which respond to HBcAg amino acids 35 - 45 and 49 - 69), cytotoxic T lymphocytes (HBcAg 18 - 27), and B cells (HBcAg 76 - 87 and 105 - 116) [11, 12]. Escape mutations within HBcAg epitopes potentially allow HBV to avoid detection and clearance by the host immune system and may represent a mechanism through which infections may persist in some patients. It is worthy to mention that the D genotype is a unique genotype of HBV present in Iran [13]. Hence, it seems that overall genotype variation is not important in the alteration of immune responses among Iranian patients and that specific mutations within the HBV genome, especially in the HBc gene, can be the main viral factor for attenuating immune responses and allowing infection to persist. Therefore, this review draws on current reports regarding HBc gene mutations of HBV isolated from different clinical presentations of Iranian patients suffering from hepatitis B to find the relationship between reported mutations and the outcome of the infection. In order to more comprehensively report the mutation status in the HBc gene, the authors have included a search of the reported gene sequences from Iran in the NCBI nucleotide Gen Bank.

Mutations in HBcAg from amino acids 18 to 27 (the cytotoxic T lymphocyte response region)

Immune responses by cytotoxic CD8 positive T cells to viral infections are the main arm of immune responses that can lead to the complete eradication of viral infections [14]. Previous studies revealed that the amino acids from 18 to 27 of HBcAg are the main region that is recognized by cytotoxic CD8 positive T cells [15]. Therefore, mutations in this region can lead to attenuated cell cytotoxicity responses and, subsequently, persistent HBV infection can occur [16]. Several researchers from Iran have evaluated the mutations in the epitope from amino acids 18 to 27 of HBcAg including our previous study on asymptomatic HBV infected patients which reported that there were no mutations found in this epitope of HBcAg [11]. However, in our search of the GenBank we found a study which reported three mutations T21S, A26S (Gene ID (GI): 54125538), and A21S (GI: 34452647) in this region in chronic hepatitis B infected patients. In addition, other mutations were reported by other researchers as follow: H21S (GI: 190351525), I27V (GI: 190351480), Q21S (GI: 190351471), G21S (GI: 190351246), N21S (GI: 190351182), Y23F (GI: 190351144), Y24F (GI: 190351144). In addition to the mentioned mutations, Mohebi et al. added another mutation, V21S (GI: 340748298) within the epitope of HBcAg. However, these mutations were also reported by other researchers, but they included the additional mutations M21S (GI: 289598367), S19L (GI: 289598275), and L27V (GI: 289598154). The frequency of mutations targeting Ser21 suggests that this amino acid plays an important role for CTL immune responses. Interestingly, Sendi et al. also demonstrated that Ser21 was the most variable amino acid and that it was substituted by seven different amino acids [16]. It is worthy to mention that the epitope from amino acids 18 to 27 is antigen-presented by HLA-A2 molecules and, hence, this region of HBcAg would typically be recognized by immune cells of HLA-A2 positive patients. In our studies we evaluated the patients with respect to HLA-A2 but no mutations were found in the HLA-A2 positive patients. Moreover, all of the studies mentioned above, with the exception of Sendi et al., did not evaluate HLA-A2 status in the studied patients. Therefore, we cannot rule out the possibility that the reason for long term infection in those reported studies was not caused by mutations in the antigen presentation genes.

Mutations in HBcAg from amino acids 35 - 45 and 49 - 69 (the T helper lymphocyte response regions)

T helper cells are the main cells responsible for cytokine production and co-stimulatory molecule expression which assists humoral and cellular immunity against viral infections [17,18]. Previous studies demonstrated that amino acids from 35 to 45 and 49 to 69 with HBcAg can be presented by type 2 MHC antigen presenting cells to T helper cells [16,19]. Activated T helper cells can help B lymphocytes to induce isotype switching and affinity maturation [20,21]. T helpers can also help T cytotoxic cells to eradicate viral infections such as HBV [22,23]. Therefore, mutations in these regions can lead to defective T helper responses and subsequently to compromised humoral and cellular immunity against HBV. Iranian researchers have reported several mutations in the amino acids from 35 to 45 and 49 to 69 of HBcAg. Our study on asymptomatic HBV infected patients showed that there were four mutations (D64E, I66M, N67T, and S69A) in amino acids from 49 to 69, while no mutations were found in amino acids from 35 to 45 [11]. According to our search of Gen Bank, Amini-Bavil-Olyaee et al. reported four mutations F38Y(GI: 54125538), A35S (GI: 34452656), S45P (GI: 34452652), and Q40E (GI: 34452640) in the 35 - 45 region and seven mutations S67T (GI: 54125538), D64E (GI: 34452658), F59I (GI: 34452654), I66M plus S69A (GI: 34452652), V55L (GI: 34452638), and T56R (GI: 34452626) in the 49 - 69 region of chronic hepatitis B infected patients. Mohebi et al. also have submitted T35S plus D40E (GI: 190351568), N45P (GI: 190351293), and T45P (GI: 190351276) mutations in the amino acids from 35 to 45 and several mutations in amino acids from 49 to 69 as follow: T49S (GI: 190351568), N67T (GI: 190351539), V59I (GI: 190351497), A50P (GI: 190351483), A50H

(GI:190351414), I55L (GI:190351403), H50P (GI: 190351377), H57Q, V58A plus S59I (GI: 190351322), Q67T (GI: 190351296), C59I (GI: 190351210), and W61C (GI: 190351175). In 2012, these researchers also submitted other HBc gene sequences with the following mutations: E38Y (GI: 340748227) and G40E (GI: 340748170) within the amino acids from 35 to 45 and E58A (GI: 340748457), I60L (GI: 340748298), V56A (GI: 340748263), and S50P (GI: 340748220) within the amino acids from 49 to 69 of HBcAg [12]. Garmiri et al. reported the existence of the same mutations but added several new ones including P35S (GI: 289598430), Q45P (GI: 289598388), H45P (GI: 289598381), P41I (GI: 289598268), and S41A (GI: 289598189) in amino acids from 35 to 45 and Y59I plus L66M plus G67T (GI: 289598409), A67T (GI: 289598402), T66M (GI: 289598261), F59L (GI: 289598212), and S60L (GI: 289598168) in amino acids from 49 to 69 [24]. F38Y and D40E are the most common mutations within the 35 - 45 region whereas D64E, S69A, T/I66M, and S/N67T are the most common in the 49 - 69 region (see Table 1). Based on these results, it can be concluded that these mutations may play key roles in the escape of T helper immune responses by HBV. Previous studies revealed that T helpers induce humoral and cellular immunity against viral infections via expression of co-stimulatory molecules and cytokines [18]. Barboza et al. reported that T helper cells of chronic HBV infected patients were unable to increase the expression of CD40L as the main co-stimulatory molecule for macrophage and B lymphocyte activation [25]. Several studies also demonstrated that cytokine production by T helper cells was decreased in hepatitis B infected patients [8,9,26]. Therefore, it may be concluded that HBV can escape recognition by T helper lymphocytes especially when they harbor the F38Y and D40E mutations in the 35 - 45 region and D64E, S69A, T/I66M, and S/N67T in the 49 - 69 region.

Mutations in HBcAg from amino acids 76 - 87 and 105 - 116 (the B cell lymphocyte response regions)

Humoral immunity is another arm of immune responses, especially in the protection against viral infection [27,28]. Anti-HBs are protective antibodies that are produced against HBsAg (HBV vaccination components) [29]. Anti-HBc is produced against amino acids 76 - 87 as well as to the 105 - 116 region of HBcAg [30]. Furthermore, humoral immunity is considered one of the main immune responses against HBV and therefore mutations within the B cell lymphocyte epitope recognition region of HBcAg can be utilized by HBV to escape from these immune responses [31,32]. Interestingly, we have shown previously that there were three mutations in the 76 - 87 region (Q77E, T80I, N87S) and one mutation in the 105 - 116 region (L116I) of asymptomatic HBV infected patients [11]. Like other HBc gene regions, our search of GenBank revealed that Amini-Bavil-Olyaee et al. found five mutations Q79P, Q80I (GI: 54125538), T80I (GI: 34452658), V80I (GI: 34452656), and M85V (GI: 34452631) in the76 - 87 region as well as E113D (GI: 54125538), L116I (GI: 34452654), T105I (GI: 34452652), and N113E (GI: 34452624) in the 105 - 116 region of chronic hepatitis B infected patients.

Additionally, other mutations were reported by Mohebi et al. which included G87S (GI: 190351556), Q77E, V114T (GI: 190351536), Q84E (GI: 190351525), A80I (GI: 190351501), E83D (GI: 190351486), I86V (GI: 190351480), P80I (GI: 190351417), Q87S (GI: 190351210), T87S (GI: 190351199), and I85V plus N87S (GI: 190351156) in the 76 - 87 region and P113E (GI: 190351556), I114T (GI: 190351539), V105I plus M109T (GI: 190351525), Q113E (GI: 190351522), A109T (GI: 190351497), V114T (GI: 190351486), V116I (GI: 190351471), S113E (GI: 190351417), L105I (GI: 190351414), T116I (GI: 190351377), S116I (GI: 190351347), S109T (GI: 190351325), K111R (GI: 190351259), and K112R (GI: 190351175) in the amino acids from 105 to 116. Mohebi et al. also reported that the mentioned mutations were also found in their HBV chronic patients. In addition, they also reported mutations including N78D (GI: 340748661), N67T (GI: 340748592), D77E (GI: 340748256), and A85V (GI: 340748185) within the 76 - 87 region and I109T (GI: 340748675), Y107C (GI: 340748585), and M119T (GI: 340748213) within the amino acids from 105 to 116 of HBcAg. Although, these mutations were also reported by Garmiri et al. they also included mutations such as R87S (GI: 289598402) and E84D (GI: 289598317) in the 76 - 87 region and M114T (GI: 289598409) and N109T (GI: 289598212) in the 105 - 116 region in their report. The most common mutations in the epitope recognized by B cell lymphocytes, in the region from 76 to 87 were those changing Ile80 (Table 1). Ise80 was replaced by five different amino acids including Glutamine, Proline, Alanine, Threonine and Valine. D/Q77E, A/I/M85V, M/I/V114T and R/T/Q/G/N87S mutations were also reported but with less frequency than mutations in position Ile80. Therefore, it appears that mutations at Glu77, Val85, Ser87, Thr114 and especially Ile80 were seen repeatedly in the B cell lymphocyte epitope recognition region of the HBc gene in HBV infected Iranian patients and these mutations may be a mechanism employed by the virus to escape antibody recognition.

CONCLUSION

According to the reports mentioned above, it seems that the prevalence of HBc gene mutations is frequent in Iranian HBV infected patients. Escape from immune responses is a plausible reason for the high prevalence of HBc gene mutations among Iranian HBV infected patients. Moreover, the studies mentioned above are in reference to the mutations within the HBc gene of

Table 1. The table reports the amino acid substitutions found in the five epitopes (CTL recognition epitope from amino acids 18 - 27, T helper recognition epitopes from 35 - 45 and 49 - 69 and B cell recognition epitopes 76 - 87 and 105 - 116) of the hepatitis B core antigen. Substitutions are classified by the epitope they are found in. Also shown are the GenBank gene identifiers and the references in which they are reported. Amino Acids are represented using the single letter code and numbers represent the position of the amino acid within the HBc antigen protein.

Status of mutation in antigenic epitopes of the HBV core protein					NCBI GenBank Gene ID	Reference
CTL (HBcAg 18 - 27)	T helper (HBcAg 35 - 45)	T helper (HBcAg 49 - 69)	B cell (HBcAg 76 - 87)	B cell (HBcAg 105 - 116)		
NF	NF	D64E I66M N67T S69A	Q77E T80I N87S	L116I	No submitted	[11]
T21S A26S	F38Y	S67T	Q79P Q80I	E113D	54125538	[34]
		D64E	T80I		34452658	[35]
	A35S	D64E	V80I		34452656	[35]
		F59I	T80I	L116I	34452654	[35]
	S45P	D64E I66M S69A	T80I	T105I D113E	34452652	[35]
A21S			T80I	L116I D113E	34452647	[35]
A21S	F38Y Q40E	D64E S67T	T80I		34452640	[35]
T21S		V55L	T80I	L116I	34452638	[35]
		D64E I66M S69A	T80I		34452636	[35]
		I66M	T80I M85V	L116I D113E	34452631	[35]
		T56R			34452626	[35]
		D64E I66M	V80I	N113E	34452624	[35]
	T35S D40E	T49S			190351568	[36]
	D40E		T80I		190351563	[36]
A21S	F38Y D40E	D64E S67T	T80I G87S	P113E	190351556	[36]
A21S	F38Y D40E			L116I	190351550	[36]
T21S		D64E N67T	T80I	I114T	190351539	[36]
		D64E I66M N67T S69A	Q77E T80I V114T		190351536	[36]
H21S	D40E	D64E N67T	Q77E T80I Q84E	V105I M109T	190351525	[36]
	F38Y		D77E T80I	Q113E	190351522	[36]
		D64E	T80I		190351515	[36]
		D64E	V80I		190351508	[36]
		D64E	V80I		190351505	[36]
		D64E	A80I		190351501	[36]

		V59I	T80I	A109T Q113E	190351497	[36]
		D64E I66M S69A	T80I		190351493	[36]
		D64E	V80I		190351490	[36]
T21S A26S		D64E N67T	V80I E83D	V114T	190351486	[36]
A21S	F38Y	A50P D64E I66M S69A	T80I	L116I	190351483	[36]
I27V	A35S D40E	A50P D64E S67T	V80I E83D I86V G87S	M109T V116I	190351480	[36]
T21S		T49S	D77E T80I	D113E	190351477	[36]
	D40E	V59I	T80I		190351474	[36]
Q21S			T80I	V116I	190351471	[36]
	D40E	D64E	D77E V80I		190351468	[36]
	F38Y				190351462	[36]
	D40E		T80I	L116I	190351459	[36]
	F38Y				190351456	[36]
		D64E	Q77E V80I	D113E	190351453	[36]
			T80I	D113E	190351450	[36]
		D64E	A80I		190351447	[36]
	D40E	T49S D64E N67T	V80I G87S	Q113E V116I	190351443	[36]
	D40E	D64E I66M S69A	T80I		190351436	[36]
		T49S	V80I	L116I	190351433	[36]
		D64E I66M S69A	T80I	Q113E	190351427	[36]
		V59I D64E	V80I	V116I	190351420	[36]
H21S		N67T	P80I	S113E L116I	190351417	[36]
	F38Y D40E	A50H		L105I D113E	190351414	[36]
T21S		I55L S67T	T80I	M109T D113E L116I	190351403	[36]
			T80I	V105I V114T	190351400	[36]
A21S		D64E I66M S69A	T80I		190351397	[36]
	D40E	T49S	Q77E T80I		190351394	[36]

		T49S D64E I66M S69A	T80I	L116I	190351391	[36]
			T80I	V114T	190351380	[36]
		H50P	T80I	D113E T116I	190351377	[36]
		D64E	T80I		190351374	[36]
				D113E	190351363	[36]
		D64E I66M S69A	T80I	L116I	190351360	[36]
	F38Y D40E	H50P	D77E T80I	L116I	190351357	[36]
Q21S	F38Y D40E		T80I	L105I D113E	190351354	[36]
				S116I	190351347	[36]
A21S		D64E I66M S69A	T80I	Q113E	190351344	[36]
		D64E I66M S69A	T80I	D113E V116I	190351341	[36]
		N67T	T80I		190351328	[36]
	D40E		T80I	S109T	190351325	[36]
		H57Q V58A S59I D64E N67T		L116I	190351322	[36]
A21S	T35S	D64E S67T	T80I	M109T V116I	190351319	[36]
T21S		T49S	D77E T80I	V105I V116I	190351312	[36]
		D64E	V80I		190351304	[36]
A21S		A50P Q67T	D77E T80I G87S	V105I V114T L116I	190351296	[36]
	F38Y D40E N45P	D64E	T80I		190351293	[36]
		F59I	T80I	L116I	190351282	[36]
	F38Y		T80I	T116I	190351279	[36]
	D40E T45P	D64E I66M S69A	D77E T80I	D113E	190351276	[36]
A21S	T35S D40E	D64E	V80I	V116I	190351273	[36]
		D64E	T80I E83D G87S		190351270	[36]
	F38Y N45P		T80I	K111R	190351259	[36]
	T35S	D64E I66M S69A	T80I	L116I	190351256	[36]
T21S	A35S	T49S I60L	T80I G87S		190351253	[36]

G21S		D64E S67T	T80I	Q113E	190351246	[36]
T21S	F38Y	T49S	T80I		190351243	[36]
T21S		D64E	V80I		190351240	[36]
		D64E N67T	V80I		190351237	[36]
		D64E S67T	T80I		190351230	[36]
		D64E I66M S69A	T80I		190351227	[36]
	A35S F38Y D40E	T49S S67T	T80I		190351223	[36]
		T49S			190351216	[36]
A21S			T80I	D113E V116I	190351213	[36]
		C59I	Q79P A80I Q87S	D113E I114T	190351210	[36]
		V59I	Q77E V80I	I114T	190351207	[36]
A21S	F38Y	T49S N67T	Q79P T80I T87S	V105I	190351199	[36]
		D64E	V80I		190351196	[36]
			V80I	L116I	190351193	[36]
			T80I	Q113E	190351189	[36]
A21S	F38Y	V59I D64E	V80I	V116I	190351185	[36]
N21S	F38Y D40E	I55L D64E	Q79P A80I	V105I	190351182	[36]
		W61C		K112R	190351175	[36]
	F38Y	D64E I66M S69A		D113E	190351171	[36]
A21S	F38Y	D64E I66M S69A	T80I	V114T L116I	190351168	[36]
T21S	D40E	T49S	Q77E V80I	V114T	190351165	[36]
		D64E	T80I		190351162	[36]
		D64E	T80I		190351159	[36]
	D40E	D64E I66M N67T S69A	Q79P T80I I85V N87S		190351156	[36]
		F59I	T80I		190351149	[36]
T21S Y23F Y24F			T80I	Q113E	190351144	[36]
	F38Y	D64E		D113E V116I	190351141	[36]
A21S			D77E T80I	Q113E V116I	190351135	[36]
			Q77E T80I	D113E V116I	190351132	[36]

T21S		D64E N67T	V80I G87S		190351129	[36]
			N78D		190351126	[36]
		D64E	T80I		190351122	[36]
	F38Y D40E	T49S D64E	T80I	I109T V116I	340748675	[12]
		D64E N67T	T80I	I114T	340748668	[12]
			N78D		340748661	[12]
			T80I	L116I	340748631	[12]
			D77E	Q113E	340748616	[12]
		V59I	Q77E V80I	I114T	340748608	[12]
			T80I T87S		340748600	[12]
		T49S	N67T T80I	Q113E	340748592	[12]
A21S	F38Y	T49S	D77E T80I	Y107C L116I	340748585	[12]
		D64E	V80I		340748578	[12]
	T35S	D64E	V80I		340748554	[12]
		D64E			340748546	[12]
		D64E	T80I I85V		340748538	[12]
		D64E	T80I G87S	V116I	340748531	[12]
	D40E	D64E I66M S69A	T80I		340748524	[12]
	A35S F38Y		T80I		340748517	[12]
		T49S	T80I		340748478	[12]
	D40E S45P	D64E	V80I	M109T D113E	340748471	[12]
		D64E I66M S69A	A80I	Q113E	340748464	[12]
		E58A N67T	T80I N87S		340748457	[12]
		E58A N67T	Q79P T80I N87S		340748450	[12]
	F38Y				340748443	[12]
	F38Y	A50P	T80I		340748436	[12]
	F38Y		T80I	T105I D113E	340748429	[12]
		T49S	T80I		340748421	[12]
	T35S F38Y	T49S	T80I	I114T	340748413	[12]
				V105I	340748405	[12]
				L105I	340748381	[12]
			T80I		340748357	[12]
		D64E			340748334	[12]
		D64E	V80I		340748319	[12]
			V80I	V116I	340748312	[12]

		C59I N67T	T80I	Q113E	340748305	[12]
V21S I27V		I55L I60L N67T		V105I	340748298	[12]
A21S	F38Y	T49S N67T	Q79P T80I T87S	V105I	340748291	[12]
T21S	D40E		Q79P T80I		340748284	[12]
			T80I T87S	L116I	340748277	[12]
		D64E	Q77E V80I	D113E	340748270	[12]
		H57Q V56A S59I D64E N67T	T80I	D113E L116I	340748263	[12]
T21S		T49S	D77E T80I	V105I D113E	340748256	[12]
	D40E T45P	D64E I66M S69A	D77E T80I	D113E	340748249	[12]
		D64E N67T	A80I	D113E V105I	340748242	[12]
		I55L V59I S67T	T80I	Q113E L116I	340748235	[12]
A21S	E38Y	A50P	T80I	L116I	340748227	[12]
		S50P	V80I T87S	L116I	340748220	[12]
	T35S	D64E I66M S69A	T80I	M119T	340748213	[12]
			T80I		340748206	[12]
G21S		T49S	T80I T87S		340748199	[12]
		T49S	T80I	Q113E	340748192	[12]
		N67T	Q77E T80I A85V	D113E	340748185	[12]
T21S		N67T	Q77E T80I A85V	D113E V116I	340748178	[12]
A21S	G40E S45P	V59I D64E I66M N67T S69A	T80I	V116I	340748170	[12]
T21S		T49S	V80I		340748163	[12]
	F38Y		D77E T80I		340748133	[12]
			T80I		340748126	[12]
	T35S	D64E	T80I	V105I L116I	340748118	[12]
		D64E S67T			340748111	[12]
	D40E	D64E	V80I		340748105	[12]

			T80I		340748098	[12]
			T80I	L116I	340748091	[12]
		T49S	V80I	L116I	340748084	[12]
H21S	D40E	S67T	T80I	T105I D113E L116I	289598452	[24]
			T80I		289598444	[24]
	D40E	F59I	T80I	M109T D113E	289598437	[24]
	P35S D40E		T80I G87S		289598430	[24]
			T80I	Q113E	289598423	[24]
V21S Y24F		D64E I66M S69A	D77E T80I	V116I	289598416	[24]
I27V		I55L Y59I L66M G67T	T80I	M114T	289598409	[24]
	F38Y	A67T	Q79P T80I R87S	V105I I109T V114T	289598402	[24]
		D64E I66M N67T S69A	Q77E T80I		289598395	[24]
	A35S D40E Q45P		T80I	V105I M109T	289598388	[24]
A21S	A35S D40E H45P	N67T		T105I D113E L116I	289598381	[24]
T21S Y24F	D40E	T49S	T80I	L116I	289598374	[24]
M21S		H50P S67T	Q79P T80I		289598367	[24]
		D64E	V80I N87S		289598360	[24]
			D77E T80I	D113E V114T	289598352	[24]
	F38Y D40E	T49S	T80I G87S	I114T	289598345	[24]
		T49S	T80I		289598338	[24]
G21S			T80I		289598331	[24]
A21S		H57Q C59I D64E N67T	T80I	Q113E V116I	289598324	[24]
	F38Y	T49S I66M S69A	T80I E84D	L116I	289598317	[24]
	F38Y	S50P D64E N67T	V80I	L105I Q113E	289598310	[24]
			T80I		289598303	[24]
		H50P	T80I	D113E	289598296	[24]
A21S		T49S	T80I		289598289	[24]

A21S Y24F			T80I	V116I	289598282	[24]
S19L H21S		D64E I66M N67T S69A	T80I	L105I	289598275	[24]
T21S	F38Y P41I		Q77E T80I		289598268	[24]
A21S		A50P D64E T66M N67T	D77E T80I	D113E L116I	289598261	[24]
	H45P	D64E	V80I	L116I	289598254	[24]
		F59I N67T	T80I	L116I	289598247	[24]
T21S		S67T	Q79P Q80I		289598240	[24]
		D64E S67T			289598233	[24]
			Q79P T80I	L116I	289598226	[24]
Y24F			D77E T80I	V116I	289598219	[24]
	D40E	F59L N67T	T80I	N109T L116I	289598212	[24]
		D64E I66M S69A	T80I		289598204	[24]
			T80I	L116I	289598196	[24]
A21S	S41A	L50P D64E	V80I		289598189	[24]
V21S			T80I	L116I	289598182	[24]
	F38Y S41A	D64E I66M S69A	T80I		289598175	[24]
	D40E	T49S S60L D64E L66M N67T S69A	Q79P T80I	L116I	289598168	[24]
H21S	D40E	D64E L66M N67T S69A	T80I	Q113E	289598161	[24]
N21S L27V	A35S F38Y	D64E	V80I I85V	L105I	289598154	[24]
	F38Y		T80I	T116I	289598147	[24]
	F38Y		D77E T80I		289598140	[24]
Q21S Y24F	T35S	S67T	Q79P T80I T87S	V105I V114T	289598133	[24]
			Q79P T80I	I114T	289598126	[24]
			T80I	T105I D113E L116I	289598119	[24]
		D64E		T105I D113E	289598112	[24]
A21S	D40E	T49S F59I N67T	A80I E83D		289598104	[24]

chronic and asymptomatic patients and, therefore, may be linked to long term HBV infectivity such as chronic and asymptomatic forms of hepatitis B infection. Therefore, designing a cohort study on acute HBV infected patients in relation to HBc gene mutations and following the outcomes of infections could help find the mutations responsible for induction of long term hepatitis B infection. Previous studies reported that the HBV genotype can affect the outcome of infection. Interestingly, the D genotype is the unique prevalent HBV genotype in Iran [13]; hence, the genotype does not appear to affect the outcome of disease in Iran, rather, and it would appear that the mutations reported here can be considered more important for disease progression. In addition, based on the high prevalence of OBI in Iranian blood donors [33], evaluation of these mutations in the long term forms of hepatitis B infection would be of immense value.

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