

Clinical significance of hepatitis B surface antigen mutants

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Abstract

Hepatitis B virus (HBV) infection is a major public health problem in many countries, with nearly 300 million people worldwide carrying HBV chronic infection and over 1 million deaths per year due to cirrhosis and liver cancer. Several hepatitis B surface antigen (HBsAg) mutations have been described, most frequently due to a single amino acid substitution and seldom to a nucleotide deletion. The majority of mutations are located in the S region, but they have also been found in the pre-S1 and pre-S2 regions. Single amino acid substitutions in the major hydrophilic region of HBsAg, called the "a" determinant, have been associated with immune escape and the consequent failure of HBV vaccination and HBsAg detection, whereas deletions in the pre-S1 or pre-S2 regions have been associated with the development of hepatocellular carcinoma. This review article will focus on the HBsAg mutants and their biological and clinical implications.

Key words: Hepatitis B virus infection; Vaccine escape; Immune escape hepatocellular carcinoma; Hepatitis B surface antigen mutants

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Core tip: Antibodies to the hepatitis B surface antigen (HBsAg) produced in response to hepatitis B virus infection or vaccination and those used in diagnostic assays to detect this antigen in serum are both directed against the "a" determinant region, common to all subtypes of the virus. Mutations occurring on the loops of the "a" determinant may be responsible for the lack of protection in immunized patients and in those individuals receiving hepatitis B immune globulin or for failed detection of HBsAg using commercial diagnostic

assays. There is growing evidence in the last decade of the association between HBsAg mutations and the development of hepatocellular carcinoma (HCC), suggesting that the pre-S1 or pre-S2 large deletions are those prevalently associated with the development of HCC. This review article will focus on the clinical impact of the various HBsAg mutants.

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INTRODUCTION

Hepatitis B virus (HBV) infection is a major public health problem in most countries, with approximately 2 billion people worldwide showing exposure to the virus, nearly 300 million carrying HBV chronic infection and over 1 million deaths per year due to HBV-related end-stage liver disease, liver cirrhosis and liver cancer^[1-5].

HBV is an enveloped Hepadnavirus with an incomplete double-stranded DNA genome of 3.2 Kb^[6]. Eight genotypes, with a distinct geographical distribution, have been identified to date. Genotype A prevails in north-western Europe and in the United States, genotypes B and C in Asia, genotype D in the Mediterranean basin, the Middle East, and India, genotype E in Western Africa, genotype F in South and Central America, genotype G in the United States and France, genotype H in Northern Latin America^[7], genotype I in Laos, Vietnam, Eastern India^[8,9] and North-Western China^[10] and genotype J in Japan^[11,12].

The worldwide prevalence of chronic HBV infection in the general population borders 5%, but it differs widely from one geographical area to another, from 0.1%-2.0% in the United States and Western Europe, from 2.0%-8.0% in Eastern Mediterranean countries and Japan, and from 5.0%-20.0% in South-Eastern Asia and sub-Saharan Africa^[1,13].

Risk factors for HBV infection include transfusion of unscreened blood, renal dialysis, sexual promiscuity, sharing or re-using syringes among injection drug users, tattooing, piercing, working or residing in a health-care setting, living in a correctional facility and long-term household or intimate non-sexual contact with an HBsAg-positive individual. In highly endemic areas the majority of hepatitis B surface antigen (HBsAg) chronic carriers acquire HBV infection at birth or in the first decade of life, whereas in countries with a low endemicity HBV transmission occurs mostly in adulthood due to unprotected sexual contact, syringe sharing or parenteral exposure to contaminated medical equipment^[7,14-17].

A vaccine against HBV became available in 1982, and ten years later the World Health Organization recommended universal vaccination of newborn babies

with the HBsAg produced by yeast cells into which the genetic code for HBsAg had been inserted. The complete vaccination schedule induces protective antibody levels in more than 95% of infants, children and young adults.

The emergence of single or multiple amino acid substitutions in the HBsAg region has been found in infants born to HBsAg-positive mothers who underwent passive/active immunoprophylaxis at the birth, in HBsAg-positive liver transplant recipients treated with hyperimmune anti-HBs immune globulin and in patients who experienced loss of HBsAg after anti-HBV therapy.

This review article focuses on the impact of HBsAg mutants on vaccine escape, failure of diagnostic tests to detect HBsAg, and on the development of hepatocellular carcinoma (HCC).

HBV VIROLOGY

Human HBV is the prototype member of the Hepadnaviridae family, which includes a variety of avian and mammalian viruses sharing similar genomic organization, organ tropisms and a unique strategy of genome replication^[14].

HBV is one of the smallest enveloped animal viruses with a diameter of 42 nmol/L consisting of an outer lipid envelope and an icosahedral nucleocapsid core composed of proteins. The nucleocapsid encloses the viral DNA and a DNA polymerase acting also as a reverse transcriptase^[17]. The outer envelope contains the embedded proteins HBsAg, pre-S1 and pre-S2 involved in the viral binding of, and entry into susceptible cells. HBV is also called "Dane particle" after the name of the researcher who first observed it on electron microscopy together with filaments and 22 nmol/L spherical bodies in the serum of infected individuals^[18]. HBV infects the hepatocytes, whereas the filaments and spherical bodies do not contain the viral DNA and do not infect the liver cells. These filaments and spherical bodies show the same HBsAg reactivity as the surface of HBV and are considered to be produced by HBsAg in excess during the life cycle of the virus^[19].

The HBV genome consists of 3200 base pairs of partially double-stranded circular DNA containing four (P, C, S and X) overlapping open reading frames (ORF) with a nucleotide diversity of $\geq 8\%$ in different genotypes^[15,16]. The P gene codes for the viral polymerase/reverse transcriptase. It has four domains: A terminal domain, which serves as a protein primer for reverse transcription of pre-genomic viral RNA, a spacer region without no apparent function, the polymerase domain, which has reverse transcription activity, and the RNase H domain, responsible for the degradation of the RNA template during reverse transcription.

The core (C) gene codes for HBcAg, the major structural protein of the nucleocapsid. The preC/C ORF is transcribed into a precore/core fusion protein. During entry into the endoplasmic reticulum, 19 amino acids are cleaved from the N-terminal end of the precore protein by a signal peptidase. When transported into the

Golgi compartment, additional amino acids are removed from the C-terminal end by intra-Golgi proteases to form the HBe antigen. This antigen, which is secreted into the serum, is used as a marker of active HBV replication in clinical practice. The possibility that the circulating HBe antigen may suppress the immune response and favor HBV replication has been hypothesized^[14] but never proven, and the biological function of this protein, if any, remains unknown. The preS/S ORF encodes the envelope proteins HBsAg, pre-S1 and pre-S2. The X gene codes for potent transactivating factors of viral and cellular genes (HBxAg), some of which possibly correlated to the development of HCC^[20,21].

HBsAg STRUCTURE AND VARIANTS

The preS/S ORF encodes three different structurally related envelope proteins, termed the large (L), middle-sized (M) and small (S) protein, that is synthesized from alternative initiation codons. The three proteins share the same carboxy-terminus but have different amino terminal extensions. In particular, the S protein corresponding to the HBsAg consists of 226 amino acids (aa), the M protein contains an extra N-terminal extension of 55 aa, and the L protein has a further N-terminal sequence of 108-119 aa compared with the M protein^[22]. The enhancer and basic core promoter regions of S region overlap with the X gene.

HBsAg is an envelope glycoprotein that is currently the primary element for diagnosis and target of immunoprophylaxis of HBV infection. The dominant epitopes of HBsAg, which are the targets of neutralizing B-cell responses, reside in the "a" determinant (aa 124-147) within the major hydrophilic region (MHR).

Several mutations in the S region have been described and those most frequently reported in the literature are listed in Table 1. In most cases, they were aa substitutions due to a single mutation, but nucleotide deletions have also been reported. The majority of mutations were located in the S region, but some mutations were also identified in the pre-S1 or pre-S2 regions. Mutations in the S region have been found in various HBV genotypes, while those in pre-S1 or pre-S2 have been frequently observed in patients with HBV genotype C (Table 1)^[23-60].

CLINICAL SIGNIFICANCE OF HBsAg MUTANTS

Some HBsAg mutants have been associated with major biological or clinical events such as immune escape, failure to detect HBsAg and the development of HCC.

HBsAg mutants associated with immune escape

The MHR region, which is exposed to the outer surface of the virion, is situated between aa 99-169 of HBsAg. The antibodies produced after HBV vaccination and those used in diagnostic assays to detect serum HBsAg are both directed against this region and, specifically, to

a cluster of B-cell epitopes, common to all subtypes of the virus, called "a" determinant and showing a two-loop structure of aa (124-147). Mutations may occur on both loops of the "a" determinant and may be responsible for a lack of protection and the occurrence of HBV infection in immunized patients (vaccine escape) or for failure to protect by the HBIG administered as a prophylactic measure or failure to detect HBsAg in diagnostic assays. Table 2 lists the studies suggesting the association of HBsAg mutants with vaccine escape or failed HBsAg detection.

In 1988 a follow-up Italian study^[61] reported that children with a strong antibody response to HBV vaccine may still become infected with HBV. This observation was confirmed in other investigations and the conclusion on this point is that this phenomenon may involve nearly 2% of children born of HBsAg-positive mothers or with other HBsAg-positive household contacts^[61,62]. More detailed analysis identified an association of vaccine escape with a point mutation from glycine to arginine at position 145 (G145R)^[61]. This G145R mutation is the vaccine-escape mutant most frequently detected^[62-68], stable over time^[61,69] and horizontally transmissible^[70,71].

He *et al*^[72] studied 176 restaurant employees before and one year after the HBV vaccination was completed. Six (3.4%) of the 176 became HBV-DNA positive after vaccination and four (2.3%) of the six showed a point mutation within the "a" determinant (Gly-145-Ala, and Ile/Thr-126-Asn/Ser).

Ngui *et al*^[73] tested 17 HBV-infected mother/infant pairs because the infants became HBV-infected despite careful passive-active HBV immunoprophylaxis. Complete concordance in the S gene sequence was identified in 15 mother/infant pairs, while in the remaining two pairs the sequence of the S gene differed: One infant harbored three nucleic acid changes (P120Q, F134Y and D144A) and the other was carrying the I126N substitution, mutations that may interfere with HBsAg/anti-HBs binding. Mismatches in the HBV S gene were also observed in 16 of 41 HBV-infected mother/infant pairs in Singapore, of whom the infants acquired HBV infection despite HBV passive/active immunization^[74].

HBsAg mutants associated with failed HBsAg detection

In 1999, Coleman *et al*^[75] demonstrated that three commercial assays did not detect serum HBsAg in patients showing mutations including G145R in the "a" determinant. Subsequently, Zhang *et al*^[76] prepared a panel containing four dilutions of an HBsAg wild-type serum, three recombinant mutants (G145R, K141E, and T131I) and one negative sample. This panel was tested for HBsAg reactivity by the laboratories of 85 blood banks using different assays. HBsAg reactivity was detected only in 19.4% of the assays in the presence of the aa substitution G145R and in 20% in the presence of the T131I or K141E mutants.

Sticchi *et al*^[77] found G145A HBsAg mutants in 8 (3.1%) of 256 HBsAg chronic carriers, alone in 5 and with other HBsAg mutations in 3 (T126I, T131A,

Table 1 Mutations reported in the hepatitis B surface antigen regions

Codon	Type mutation	Mutation	Phenotypic consequence	HBV genotype	Ref.
Wt ¹ atg, Mt ¹ acg, Wt ^{2/3} tac, Mt ² tgc, Mt ³ cac, Wt ⁴ ttt, Mt ⁴ ttg	AAS	M197T ¹ , Y206C ² /H ³ , F220L ⁴	Low serum HBV DNA	D	[23]
Wt ¹ gga Mt ¹ gca	AAS	G145A ¹	Immune escape ¹ Lamivudine resistance ¹	A-B-C-D	[24,31,47]
Wt tgc	AAS	G145R	Immune escape	A, B-C-D	[28,35,36,40,41,45,47-50]
Mt tgg	AAS	C121W	Immune escape	A	[25]
Wr tgc, Mt tgg	AAS	C147W	Immune escape	D	[25,26]
Wt act, Mt att	AAS	T189I	Immune escape, reducing HBsAg detection signal	E-A	[27,28,33]
WT act, Mt aat	AAS	I126N	Immune escape	C	[29]
WtCaa, Mt cga	AAS	Q129R	Vaccine escape	B	[30,34]
Wt ttc, Mt tac	AAS	F161Y	Immune escape	C	[31,51]
Wt atg, Mt atc	AAS	M103I	Immune escape	D	[24,52]
Wt ttg, Mt tcg	AAS	L94S	HCC	D	[32]
WT gac, Mt gaa	AAS	D144E	Immune escape	D, C, A	[28,33,40,52]
Wt cct, Mt Tct	AAS	P127S	Immune escape	B, D	[33,34,39]
Wt act, Mt agt	AAS	T126S	Immune escape	B	[33,34,38]
WT act, MT atc	AAS	T126I	Immune escape	C, D	[28,34,53]
WT act, Mt agc	AAS	T143S	Immune escape	C, A	[34,53]
WTcca, Mt aca	AAS	P120T	Immune escape	B, D	[34,53]
Wt gca, Mt Gta	AAS	A184V	Immune escape	E	[27]
Wt gta, Mt gca	AAS	V184A	Immune escape	E	[27]
Wt Tcg, Mt acg	AAS	S143t	Immune escape	E	[25,27,53]
Wt tgt, Mt ttc	AAS	C76F	Immune escape	E	[27]
Wt cct, Mt act	AAS	P70T	Immune escape	E	[27]
Wt ata, Mt act	AAS	I82T	Immune escape	E	[27]
Wt att, Mt ctt	AAS	I110L	Immune escape	A, C	[25,53]
Wt tat, Mt ttt	AAS	Y134F	Immune escape	D	[25,55]
Wt agt, Mt aac	AAS	S207N	Immune escape	D	[25,56]
Wt tat, Mt cat	AAS	Y134H	Immune escape	D	[52]
Wt acg, Mt aat	AAS	T125N	Increased HBsAg reactivity in immunological diagnostic assays	D	[43]
Wt atg, Mt atc, WT aag, Mt agg	AAS	M103I-K122R	Immune escape	A-C-D	[24]
Wt tat, Mt tgt	AAS	Y100C	Immune escape	B-C	[45]
Wt ccc, Mt ctc, MtQ caa, MtS tcg, Mt acc	AAS	P120L/Q/S/T	Immune escape	B-C	[45]
Wt tcg, Mt cta	AAS	S143L	Immune escape	D	[52]
Wt ctt, Mt cct	AAS	L127P	Immune escape	E	[27]
Wt cct, Mt ctt	AAS	P127L	Immune escape	A	[27]
Mutations in pre-S1 region					
Wt tct, Mt aca	AAS	S98T	Significant association with disease progression (LF, LC, HCC)	D	[57]
Wt aac, Mt act	AAS	N48T	Reduced HBsAg detection signals	C	[27]
Wt cag, Mt cct	AAS	Q82P	Reduced HBsAg detection signals	C	[27]
Wt acc, Mt aat	AAS	T97N	HBsAg not detected	C	[27]
Wt aat, Mt acc	AAS	N97T	HBsAg not detected	E	[27]
Wt cct, Mt cag	AAS	P93Q	HBsAg not detected	E, C	[27]
Deletion size (bp) 39	D	Region (nt) 3046-3084	Progression to advanced liver disease	C	[58]
Deletion size (bp) 108	D	Region (nt) 2959-3066	Progression to advanced liver disease	C	[58]
Deletion size (bp) 39	D	Region (nt) 3046-3084	Progression to advanced liver disease	C	[58]
Deletion size (bp) 108	D	Region 2959-3066	Progression to advanced liver disease	C	[58]
104 th codon	AAS	Q104Stop	HCC development and immune escape	C	[59]
preS1 start	D	Not specified	HCC development and immune escape	C	[59]
Wt ucc, Mt gcc	AAS	S17A	Immune escape	C	[59]
Wt cct, Mt ctt	AAS	P32L	Immune escape	C	[59]
Wt tgg, Mt ctg, Mt agg	AAS	W43L/R	Immune escape	C	[59]
104 th codon	AAS	Q104Stop	HCC development and immune escape	C	[59]
Mutations in pre-S2 region					
Not specified	AAS	preS2-W3Stop	Immune escape	C	[59]
From 8 th codon to 23 rd codon	D		Immune escape	C	[59]

AAS: Amino acid substitution; D: Deletion; HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma; HBsAg: Hepatitis B surface antigen; LC: Liver cirrhosis; LF: Liver fibrosis.

Table 2 Studies on hepatitis B surface antigen mutations associated with immune escape and failure to detect hepatitis B surface antigen

Ref.	Country	No. of patients	Type of study	Patients with HBsAg mutation, <i>n</i> (%)	Clinical significance	HBsAg mutation
Sticchi <i>et al</i> ^[77]	Italy	256	Cross-sectional	8 (3.1)	Detection failure; vaccine escape	G145R, T126I
Luongo <i>et al</i> ^[79]	Italy	1	Case report	1	Vaccine escape	M125T, T127P Q129H
Lee <i>et al</i> ^[66]	South Korea	1	Case report	1	Vaccine escape	G145R, P120Q, I126T
Seddigh-Tonekaboni <i>et al</i> ^[68]	United Kingdom	4		2	Vaccine escape	P142S, G145R, G145A
Ngui <i>et al</i> ^[73]	England, Wales	17	Cross-sectional	2 (12)	Vaccine escape	P120Q, F134Y, D144A, I126N
Carman <i>et al</i> ^[62]	Multinational	32	Cross-sectional	1	Vaccine escape	G145R
Laoi <i>et al</i> ^[78]	Ireland	32	Cross-sectional	6 (18.5)	Vaccine escape, detection failure	G145A, F134L, D144E, S143L
Foy <i>et al</i> ^[80]	United States	1	Case report	1	Immune escape	D144E

HBsAg: Hepatitis B surface antigen.

C139Y, E/D144G, T126I, M133L, P120Q or T126I). In the three patients with a multiple mutation, HBsAg was undetectable by 3 of 5 routine assays used in this study.

HBsAg mutants associated with failure to detect HBsAg have also been observed in patients with acute hepatitis B^[78-80]. Laoi *et al*^[78] studied 32 consecutive patients with acute hepatitis B and found a single or multiple amino acid substitution in 6 (18.5%) isolates. The G145A substitution along with the F134L were responsible for failure to detect HBsAg in one of these 6 and the D144E and S143L in another two isolates, whereas the other mutants identified (R113Thr, Ser114-Pro, Thr118Val, Ala128Val) were of unclear significance.

HBsAg mutations associated with HCC development

The HBsAg mutations prevalently associated with the development of HCC are the large deletions involving the pre-S1 or pre-S2 regions^[81]. These deletions naturally occur during the chronic phase of HBV infection and induce the synthesis of truncated variants of the large envelope protein, with important immunological and clinical consequences^[82]. In fact, these variants present reduced antigenicity and, by altering the immune response, may favour the replicative activity of the virus^[83]. In addition, the pre-S deletions decrease the expression of middle and small surface proteins, resulting in intracellular accumulation of viral particles that may induce stress in the endoplasmic reticulum, oxidative DNA damage and genomic instability, and possibly lead to a higher rate of neoplastic transformation^[84]. The growing evidence on the association between HBsAg mutations and the development of HCC emerging in the last decade is shown in Table 3.

In 2003, Huy *et al*^[85] conducted a multicenter cross-sectional study on 352 HBsAg-positive patients from 12 countries in five continents or subcontinents and demonstrated a higher prevalence of pre-S1 and/or pre-S2 deletions and pre-S2 start codon mutations in patients with HCC than in those without (35.7% vs 16.5%, $P <$

0.05). In accordance with this, a correlation between a pre-S deletion and the presence of liver cirrhosis or HCC was described in an observational Japanese study^[86]. Also, in a cross-sectional Italian study, the prevalence of pre-S2 deletions or start codon mutations was much higher in the 19 patients with HBV-related HCC than in 91 HBV carriers without HCC (84.2% vs 43.9%, $P < 0.02$)^[87]. In 2006, Chen *et al*^[88] found a higher prevalence of pre-S deletions in 50 Taiwanese patients with HBV-related hepatocellular carcinoma than in 102 HBV-infected individuals without HCC (52.0% vs 29.4%, $P < 0.0001$). Similar data come from three studies performed in Taiwan^[89], South Korea^[90] and China^[91], respectively. In addition, a South Korean study performed by Mun *et al*^[92] demonstrated a correlation of both pre-S1 deletions and pre-S1 start codon mutations with the occurrence of HCC ($P = 0.027$ and $P = 0.048$, respectively); in this study the presence of pre-S2 deletions was also significantly associated with the development of liver cirrhosis ($P = 0.001$). The association of pre-S deletions and pre-S2 start codon mutations with the presence or the development of HCC was confirmed in other studies performed in southern Asia^[93-95]. Moreover, a cross-sectional South Korean study on 119 HBsAg-positive patients^[96] showed a higher prevalence of pre-S1 deletions in patients with HCC than in those without. In a case-control study^[97] on 192 HBsAg-positive patients from Taiwan, the pre-S2, but not pre-S1 deletions, were associated with the occurrence of HCC, data endorsed by the results of a subsequent South Korean case-control study on 270 HBV-infected patients^[98] that described a correlation between pre-S2 but not pre-S1 deletions or pre-S2 start codon mutations and HCC. The pre-S deletions were significantly associated with the development of HCC also in a study performed by Kao *et al*^[99] on 168 HBV chronic patients from Taiwan.

A prospective study performed in South Korea investigated 195 patients with chronic HBV infection^[100] and showed a higher incidence of HCC in those who

Table 3 Clinical significance of hepatitis B surface antigen mutations in chronic hepatitis B

Ref.	Country	No. of patients	Type of study	Patients with HBsAg mutation, <i>n</i> (%)	Clinical significance
Abe <i>et al</i> ^[103]	Japan	40	Case-control	27/30 (90) in HCC+ 0/10 (0) in HCC-	Correlation between pre-S1-S2 deletion and HCC ($P < 0.001$)
Bläckberg <i>et al</i> ^[106]	Sweden	35	Case-control	8/16 (50) in HCC+ 4/19 (21) in HCC-	No correlation between Pre-S2 mutations and HCC ($P > 0.05$)
Cao <i>et al</i> ^[94]	China	97	Case-control	34/47 (72.3) in HCC+ 13/50 (26) in HCC-	Correlation between pre-S deletion or pre-S2 start codon mutation and HCC ($P < 0.001$)
Chen <i>et al</i> ^[88]	Taiwan	152	Cross-sectional	26/50 (52.0) in HCC+ 30/102 (29.4) in HCC-	Correlation between pre-S deletion and HCC ($P < 0.001$)
Chen <i>et al</i> ^[95]	Taiwan	240	Case-control	Pre-S deletion: 28/80 (35) in HCC+ <i>vs</i> 27/160 (16.9) in HCC-	Correlation between pre-S deletion ($P = 0.002$), W4P/R ($P = 0.021$) and M1V/I/A mutations ($P = 0.011$) and HCC
Choi <i>et al</i> ^[90]	South Korea	300	Cross-sectional	W4P/R: 10/80 (12.5) in HCC+ <i>vs</i> 7/160 (4.4) in HCC- M1V/I/A: 23/80 (28.8) in HCC+ <i>vs</i> 24/160 (15) in HCC-	Correlation between pre-S deletion or pre-S2 start codon mutation and HCC ($P < 0.001$)
Fang <i>et al</i> ^[93]	China	66	Case-control	51/228 (22.4) in HCC- 15/33 (45.5) in HCC+	Correlation between pre-S deletion and HCC ($P < 0.01$)
Gao <i>et al</i> ^[91]	China	79	Cross-sectional	6/33 (18.2) in HCC- 10/26 (38.5) in HCC+	Correlation between pre-S deletion and HCC ($P = 0.001$)
Huang <i>et al</i> ^[104]	Taiwan	38	Case-control	3/53 (5.7) in HCC- 9/19 (47.4) in HCC+	Correlation between pre-S deletion and HCC ($P = 0.008$)
Hung <i>et al</i> ^[110]	Taiwan	313	Cross-sectional	1/19 (5.3) in HCC- 41/146 (40) in HCC+	Correlation between pre-S deletion and HCC ($P < 0.001$)
Huy <i>et al</i> ^[85]	12 countries	352	Cross-sectional	5/167 (3.0) in HCC- 17/49 (34.7) in HCC+ 50/303 (16.5) in HCC-	Correlation between pre-S1/S2 deletion and pre-S2 start codon mutations and HCC ($P < 0.05$)
Jang <i>et al</i> ^[96]	South Korea	119	Cross-sectional	17/48 (35.4) in HCC+ 13/71 (18.3) in HCC-	Correlation between pre-S deletion and HCC ($P < 0.05$)
Kao <i>et al</i> ^[99]	Taiwan	168	Case-control	56/112 (50.0) in HCC+ 4/56 (7.1) in HCC-	Correlation between pre-S deletion and HCC ($P < 0.001$)
Lee <i>et al</i> ^[98]	South Korea	270	Case-control	28/135 (18.5) in HCC+ 6/135 (4.4) in HCC-	Correlation between pre-S2 deletion and HCC ($P < 0.001$)
Lee <i>et al</i> ^[107]	South Korea	247	Cross-sectional	19/153 (12.4) in advanced liver disease (LC or HCC) 1/94 (1.1) in non-advanced liver disease	Correlation between W4P/R mutation and HCC or cirrhosis ($P < 0.05$)
Lin <i>et al</i> ^[89]	Taiwan	266	Cross-sectional	19/64 (29.7) in HCC+ 25/202 (12.4) in HCC-	Correlation between pre-S deletion and HCC ($P = 0.02$)
Mun <i>et al</i> ^[92]	South Korea	120	Cross-sectional	Pre-S1: 13/40 (32.5) in HCC+ <i>vs</i> 11/80 (13.7) in HCC- Pre-S1 start codon: 9/40 (22.5) in HCC+ <i>vs</i> 4/80 (5.0) in HCC- Pre-S2: 8/21 (38.1) in LC+ <i>vs</i> 4/59 (6.8) in LC-	Correlation between pre-S1 ($P = 0.027$) and pre-S1 start codon mutations ($P = 0.048$) and HCC. Correlation between pre-S2 deletions and cirrhosis ($P = 0.001$)
Qu <i>et al</i> ^[102]	China	193	Case-control	Pre-S deletion: 28/96 (29.2) <i>vs</i> 11/97 (11.3), Pre-S2 start codon: 17/96 (17.7) <i>vs</i> 7/97 (7.2), T31C: 23/96 (24.0) <i>vs</i> 37/97 (38.1), T53C: 36/96 (37.5) <i>vs</i> 23/97 (23.7), T766A: 13/96 (13.5) <i>vs</i> 14/97 (14.4) in HCC+ <i>vs</i> HCC-	Correlation between pre-S deletion ($P = 0.003$), pre-S2 start codon ($P = 0.027$), T31C ($P = 0.044$), T53C ($P = 0.027$) but not T766A mutation ($P = 0.966$) and HCC
Raimondo <i>et al</i> ^[87]	Italy	110	Cross-sectional	16/19 (84.2) in HCC 40/91 (43.9) in HCC-	Correlation between pre-S2 deletion or start codon mutation and HCC ($P < 0.02$)
Sinn <i>et al</i> ^[100]	South Korea	195	Cohort	13/24 (54.2) in HCC+ 31/171 (18.1) in HCC-	Correlation between pre-S mutation and HCC ($P < 0.001$)
Sugauchi <i>et al</i> ^[86]	Japan	160	Cross-sectional	20/58 (34.5) in advanced liver disease (LC or HCC) 17/102 (16.7) in non-advanced liver disease	Correlation between pre-S deletion and HCC or cirrhosis ($P < 0.05$)
Sung <i>et al</i> ^[108]	Hong Kong	26	Case-control	T31C: 6/16 (37.5) in HCC+ <i>vs</i> 0/10 (0.0) in HCC- T53C: 6/16 (37.5) in HCC+ <i>vs</i> 1/10 (10.0) in HCC-	Correlation between T31C and T53C mutations and HCC ($P < 0.05$)
Thongbai <i>et al</i> ^[105]	Thailand	154	Cross-sectional	24/65 (36.9) in HCC+ 34/89 (38.2) in HCC-	No correlation between pre-S1/S2/S deletion or start codon mutation and HCC ($P > 0.1$)
Yeung <i>et al</i> ^[97]	Taiwan	192	Case-control	28/96 (29.2) in HCC+ 14/96 (14.6) in HCC-	Correlation between pre-S deletion and HCC ($P = 0.015$)
Zhao <i>et al</i> ^[101]	China	317	Case-control	74/157 (47.1) in HCC+ 45/160 (28.1) in HCC-	Correlation between pre-S deletion and HCC ($P < 0.001$)

Zhu <i>et al</i> ^[109]	China	55	Case-control	4/20 (20.0) with Pre-S2 start codon, 5/20 (25.0) with T53C and 3/20 with T766A in HCC+ vs 0/20 in HCC-	Correlation between pre-S2 start codon ($P = 0.014$), T53C ($P = 0.004$) and T766A mutation ($P = 0.043$) and HCC
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HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma; HBsAg: Hepatitis B surface antigen; LC: Liver cirrhosis.

tested positive for pre-S mutations. Two subsequent Chinese case-control studies on 317 and 193 HBsAg-positive patients, respectively, identified pre-S deletions^[101,102] and pre-S2 start codon mutations^[102] as independent predictors of HCC development. Abe *et al*^[103] found a correlation between the presence of pre-S1 or pre-S2 deletions and the occurrence of HCC in a case-control study on 40 Asian children with chronic HBV infection, a finding confirmed in a retrospective study on 38 Taiwanese children^[104] in which the presence of pre-S mutations was identified as an independent predictor of HCC development.

Instead, a cross-sectional study^[105] enrolling 154 patients from Thailand failed to show an association between HCC and pre-S1 or pre-S2 deletions or start codon mutations. Likewise, a small case-control study showed no association between pre-S2 mutations and HCC in 35 patients from different countries^[106].

The S region of the HBV genome may present point mutations that could alter HBsAg secretion. These point mutations were investigated by some Authors to identify a possible correlation between their presence and the development of HCC. Chen *et al*^[95] found a correlation between the W4P/R mutation and the occurrence of HCC, an observation endorsed by the data from a cross-sectional study from South Korea^[107] on 247 HBsAg-positive patients in which the prevalence of W4P/R mutants was higher in patients with cirrhosis or HCC than in those with a less severe liver illness. In addition, Qu *et al*^[102] in a larger study confirmed the association of the T31C and T53C mutations with the occurrence of HCC previously demonstrated in a small cohort study^[108] published in 2008. In Qu's study the T766A mutant and HCC were not associated, whereas a case-control study carried out by Zhu *et al*^[109] on 55 HBV-infected Chinese patients showed a significant association between the pre-S2 start codon ($P = 0.014$), T53C ($P = 0.004$) and T766A ($P = 0.043$) mutations and the occurrence of HCC.

CONCLUSION

The association of single or multiple aa substitutions in the HBsAg region with failed protection in infants who received passive/active prophylaxis and in HBsAg-positive liver transplant patients undergoing continuous passive immunoprophylaxis should alert clinicians to the possible onset of acute hepatitis B or a reactivation of a previous HBV infection, respectively, in these cases.

Similarly, the possibility that some subjects resulting HBsAg-negative may harbor HBV infection because an aa substitution has made the presence of HBsAg undetectable with the commercially available assays

should be taken into account by clinicians and healthcare personnel working in laboratories and blood banks.

Although several studies reported an association between HBsAg mutations and HCC, the data on this point are not conclusive because most of the studies were performed in south-eastern Asia, some of them were very small, most of them were cross-sectional and a few reported data contrasting with those from the majority of studies. A large worldwide study, planned on the basis of the data available, would almost certainly improve our knowledge on this topic.

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