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Frequency of hepatitis B surface antigen variants (HBsAg) in hepatitis B virus genotype B and C infected East- and Southeast Asian patients: Detection by the Elecsys® HBsAg II assay

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ABSTRACT

Background: To avoid false negative results, hepatitis B surface antigen (HBsAg) assays need to detect samples with mutations in the immunodominant 'a' determinant region, which vary by ethnographic region.
Objective: We evaluated the prevalence and type of HBsAg mutations in a hepatitis B virus (HBV)-infected East- and Southeast Asian population, and the diagnostic performance of the Elecsys® HBsAg II Qualitative assay.
Study design: We analyzed 898 samples from patients with HBV infection from four sites (China [Beijing and Guangzhou], Korea and Vietnam). HBsAg mutations were detected and sequenced using highly sensitive ultra-deep sequencing and compared between the first (amino acids 124–137) and second (amino acids 139–147) loops of the 'a' determinant region using the Elecsys® HBsAg II Qualitative assay.
Results: Overall, 237 distinct amino acid mutations in the major hydrophilic region were identified; mutations were present in 660 of 898 HBV-infected patient samples (73.5%). Within the pool of 237 distinct mutations, the majority of the amino acid mutations were found in HBV genotype C (64.8%). We identified 25 previously unknown distinct mutations, mostly prevalent in genotype C-infected Korean patients (n = 18) followed by Chinese (n = 12) patients. All 898 samples were correctly identified by the Elecsys® HBsAg II Qualitative assay.
Conclusions: We observed 237 distinct (including 25 novel) mutations, demonstrating the complexity of HBsAg variants in HBV-infected East- and Southeast Asian patients. The Elecsys® HBsAg II Qualitative assay can reliably detect HBV-positive samples and is suitable for routine diagnostic use in East and Southeast Asia.

1. Background

Nearly 250 million people worldwide have chronic hepatitis B virus (HBV) infection, which can lead to long-term complications, such as cirrhosis, liver failure and hepatocellular carcinoma [1,2]. HBV is differentiated into a number of genotypes, which are characterized by

Abbreviations: HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; MHR, major hydrophilic region

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Table 1
Most frequent MHR mutations (occurring in ≥5 patient samples) in the East- and Southeast Asian cohort by HBV genotype (A–G).

Location of mutation	HBV Genotype							
	Sum	A	B	C	D	E	F	G
K122R	201	0	186	15	0	0	0	0
I126T	187	0	0	187	0	0	0	0
I126S	97	0	0	97	0	0	0	0
Y161F	55	0	55	0	0	0	0	0
M133T	46	0	12	34	0	0	0	0
T131N	41	0	6	35	0	0	0	0
T140I	41	0	39	2	0	0	0	0
R160K	38	0	0	38	0	0	0	0
A159V	35	0	13	22	0	0	0	0
G145R	34	0	6	28	0	0	0	0
Q101R	31	0	3	28	0	0	0	0
T126A	29	0	29	0	0	0	0	0
I110L	28	0	28	0	0	0	0	0
P127T	28	0	19	9	0	0	0	0
V168A	26	0	5	20	0	0	0	1
Y100C	26	0	3	23	0	0	0	0
L109P	21	0	16	5	0	0	0	0
I126V	20	0	0	20	0	0	0	0
Q101K	18	0	0	18	0	0	0	0
P120T	18	0	6	12	0	0	0	0
M133L	17	0	14	3	0	0	0	0
P120S	17	0	15	2	0	0	0	0
T113S	16	0	0	16	0	0	0	0
I126N	15	0	0	15	0	0	0	0
T123A	15	0	8	7	0	0	0	0
T123N	14	0	0	14	0	0	0	0
M103I	14	0	0	14	0	0	0	0
G145A	14	0	0	14	0	0	0	0
Q101H	13	0	1	12	0	0	0	0
Y100S	12	0	4	8	0	0	0	0
T125M	12	0	1	11	0	0	0	0
E164G	12	0	10	2	0	0	0	0
L110I	12	0	0	12	0	0	0	0
S114A	11	0	1	10	0	0	0	0
G130R	11	0	1	10	0	0	0	0
F170S	11	0	1	10	0	0	0	0
F134I	10	0	2	8	0	0	0	0
F134L	10	0	1	9	0	0	0	0
T116N	10	0	1	9	0	0	0	0
A166V	10	0	6	4	0	0	0	0
M133I	9	0	2	7	0	0	0	0
T126S	8	0	8	0	0	0	0	0
G130N	8	0	0	8	0	0	0	0
Q129N	8	0	1	7	0	0	0	0
W156G	8	0	0	8	0	0	0	0
S114T	7	0	2	5	0	0	0	0
G145E	7	0	0	7	0	0	0	0
F134S	7	0	3	4	0	0	0	0
T118K	7	0	0	7	0	0	0	0
T131P	7	0	1	6	0	0	0	0
E164D	7	0	0	7	0	0	0	0
T140S	7	0	1	6	0	0	0	0
T131I	6	0	1	5	0	0	0	0
Y100F	6	0	4	2	0	0	0	0
S167L	6	0	3	3	0	0	0	0
F134V	6	0	1	5	0	0	0	0
G130E	6	0	1	5	0	0	0	0
Q129H	6	0	5	1	0	0	0	0
W156L	6	0	2	4	0	0	0	0
Q129R	6	0	4	2	0	0	0	0
Y161S	6	0	6	0	0	0	0	0
S154P	6	0	1	5	0	0	0	0
P111L	6	0	0	6	0	0	0	0
A128V	5	0	0	5	0	0	0	0
S143T	5	0	0	5	0	0	0	0
S113T	5	0	4	0	1	0	0	0
T113N	5	0	0	5	0	0	0	0
T143M	5	0	5	0	0	0	0	0
T116A	5	0	0	5	0	0	0	0
C147Y	5	0	0	5	0	0	0	0
S136F	5	0	0	5	0	0	0	0
D144E	5	0	0	5	0	0	0	0
S136Y	5	0	0	5	0	0	0	0
G119R	5	0	1	4	0	0	0	0

differences of > 8% in genome sequence; 10 HBV genotypes (A–J) have been identified to date, with distinct geographic distributions [3]. For example, genotype C is primarily found in South-East Asia, and genotype I has been reported in Vietnam and Laos [3]. In China, genotype B is more prevalent in the south of the country, whereas genotype C is more prevalent in the north [4,5]. These genotypes are clinically important, as they are associated with differences in genetic mutations (defined herein as amino acid substitutions that differ from the genotype-specific consensus sequence), disease progression and treatment response [3].

HBV is transmitted via blood and other bodily fluids and so can threaten the safety of blood transfusion services, particularly in countries where HBV is endemic [6,7]. The hepatitis B surface antigen (HBsAg) is the primary serologic marker for HBV detection [8,9]. The majority of HBsAg immunoassays target the immunodominant 'a' determinant region, which is located in the major hydrophilic region (MHR) and is divided into two loops of amino acid residues (the first loop comprises amino acids 124–137; the second loop comprises amino acids 139–147) [8,10]. Commercially available HBsAg assays must be able to accurately detect samples with known mutations in the 'a' determinant region, such as the common vaccine-induced immune-escape variant G145R [8], to avoid false-negative results [10–12].

In a recent global study of 1553 HBsAg-positive blood samples from 20 countries across Africa, America, Asia and Europe, we observed HBsAg MHR mutations in 72.8% of sequenced samples, three times higher than previously reported. The highest prevalence of mutations in the 'a' determinant region was observed among samples from Asia [13]. We also demonstrated that the Elecsys® HBsAg II Qualitative assay can reliably detect HBV-positive samples with known *in vivo* mutations in the 'a' determinant region.

2. Objectives

We sought to evaluate the prevalence of HBsAg mutations in an East- and Southeast Asian chronic HBV-infected population. This consisted of Korean and Vietnamese patients from the global study [13], and a new cohort of patients from China. A further objective was to determine the diagnostic performance of the Elecsys® HBsAg II Qualitative assay in these patients. This East- and Southeast Asian population was chosen due to the high rates of endemic HBV infection in the region; a systematic review of articles published between 1965 and 2013 reported an estimated prevalence of HBsAg seropositivity in China, Korea and Vietnam of 5.49%, 4.36% and 10.79%, respectively, compared with a global prevalence of 3.61% [1].

3. Study design

The global study included samples from 1553 patients with HBV infection obtained between January 2007 and February 2015 (Africa, n = 435; Asia, n = 653; Europe, n = 72; Latin America, n = 35; Middle East, n = 79; USA, n = 234; unknown, n = 45) excluding the samples (n = 407) collected in China [13]. Of these, 562 samples were from patients with chronic HBV infection (diagnosed with HBV > 6 months before enrollment) and 991 samples were defined as unselected random HBV (samples with HBV DNA [> 100 IU/ml] or HBsAg positivity obtained from clinical settings in Europe, South Africa and the USA). The