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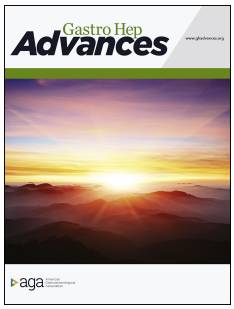

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PII: S2772-5723(23)00017-1

DOI: https://doi.org/10.1016/j.gastha.2023.01.017

Reference: GASTHA 247

To appear in: *Gastro Hep Advances*

Received Date: 31 October 2022

Revised Date: 18 January 2023

Accepted Date: 24 January 2023

Please cite this article as: Thi Cam Huong N, Vu HA, Luong BA, Makram AM, Elsheikh R, Huy NT, Le Hoa PT, The coexistence of hepatitis B surface antigen and anti-HBs in patients with chronic HBV infection: prevalence and related factors, *Gastro Hep Advances* (2023), doi: https://doi.org/10.1016/j.gastha.2023.01.017.

The coexistence of hepatitis B surface antigen and anti-HBs in patients with chronic HBV infection: prevalence and related factors

Running title: Coexistence of HBsAg and anti-HBs

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Abbreviations

- **Anti-HBs:** antibody to hepatitis B surface antigen
- **CHB:** chronic hepatitis B
- **HBeAg:** hepatitis B e antigen
- **HBsAg:** hepatitis B surface antigen
- **HBV:** hepatitis B virus
- **HCC:** hepatocellular carcinoma
- **HCM:** Ho Chi Minh City
- **MHR:** major hydrophilic region

Declarations

Conflicts of interest

The authors disclosed no conflicts.

Funding

The funding for conducting anti-HBs test for all of stored serum samples in this research is supported by the Faculty of Genetic Immunity, Nagasaki University Japan. The Sequencing of the S gene was supported by the grant of research from University of Medicine and Pharmacy at Ho Chi Minh City.

Ethical Statement

The corresponding author, on behalf of all authors, jointly and severally, certifies that their institution has approved the protocol for any investigation involving humans or animals and that all experimentation was conducted in conformity with ethical and humane principles of research.

Reporting Guidelines

Helsinki Declaration, SAGER

Data Transparency Statement

The data obtained for the conduction of this research project are available through the references listed. And the data used for the analysis can be easily obtained by contacting the corresponding author (NTH) or the first author (NTCH).

Authors' contribution

NTCH, PTLH, HAV, BAL, NTH accounted for the idea, collected the data, and performed the data analysis. NTCH, BAL and HAV were responsible for the data visualization and tables formation. All authors contributed to writing the manuscript and approved the final version before submission for publication. The research project was overseen by NTH, PTLH.

Acknowledgments

The researchers would like to express their gratitude to all patients who participated in this study. We thanks medical staff of Center for Molecular Biomedicine, University of

Medicine and Pharmacy at Ho Chi Minh City who stored the sample, sequencing the S regions and analyzed the mutations.

Abstract

Background and aims:

The prevalence of coexistence of HBsAg and anti-HBs in chronic HBV-infected patients is different between studies. The mutations on the S gene were proved as the cause of this coexistence. This study determined the frequency and factors associated with coexistence of HBsAg and anti-HBs in chronic HBV-infected patients.

Methods:

This cross-sectional study was conducted at University Medical Center at Ho Chi Minh City, Vietnam, from 4/2014 to 12/2020. HBeAg, HBsAg, and anti-HBs were measured by chemiluminescent immunoassay. Mutations on the HBV small S gene from amino acids 1 to 227 were detected using Sanger sequencing on 177 patients.

Results:

A total of 521 chronic HBV-infected patients were enrolled, including 350 males (62.7%), 17.1% with hepatic fibrosis of ≥F3 and 9.8% with HCC. The coexistence of HBsAg and anti-HBs was detected in 9.8%, with 17.9% among genotype C compared to 7.4% in genotype B, p=0.001. The coexistence group had lower levels of HBsAg titers (p=0.052). There were significantly higher rates of coexistence in the group with HCC (19.6% vs. 8.7%, p=0.013). The existence of point mutations on the major hydrophilic region and the "a" determinant region of HBV was more frequently detected in the HBsAg and anti-HBs coexistence group (p=0.043, p=0.008, respectively).

Conclusions: The coexistence of HBsAg and anti-HBs was detected more frequently in the HBV genotype C group. The coexistence status was related to lower HBsAg

87 titers, mutations on the MHR, and/or the “a” determinant and exposed significant
88 relation with HCC.

89 **Keywords:** Hepatitis B virus; Hepatitis B surface antigen; anti-hepatitis B surface,
90 Hepatocellular carcinoma; Major hydrophilic region

91

Introduction

Acute viral hepatitis B (HBV) infection often results in HBsAg loss and anti-HBs seroconversion exhibiting complete viral clearance and immune protection for further exposure. In contrast, in chronic infection, HBV is not eradicated, leading to prolonging the existence of HBsAg, and the mutually exclusive anti-HBs is not detected [1, 2]. In chronic HBV-infected people, HBsAg clearance appears less than 2% per year, but the progression to anti-HBs seroconversion slowly occurs months or years afterward or never be observed [3].

The coexistence of HBsAg and anti-HBs in chronic hepatitis B (CHB) patients is an entity and has been of interest since 1970s'. In the coexistence patients, anti-HBs had been recorded with a low affinity to HBsAg. The underlying causes of the coexistence of anti-HBs in CHB patients remained unclear for decades. However, in CHB with anti-HBs coexistence patients who had been HBV vaccinated or immunoglobulin treated, mutations in S genes were detected. This finding suggested that the selection of mutated S variants (known as "vaccine escape mutants") helps those variants escape the neutralizing activity of anti-HBs [4].

HBsAg contains several antigenic epitopes in which the "a" determinant spans the amino acids 124-147 in the S gene [5]. Mutations in the "a" determinant region result in the antigenicity alteration of the HBsAg. Some common mutations that escape immunity by amino acid replacements are G145R, sD144A, sP142S, sQ129H, sI/T126N/A, and sM133L. Experiments have confirmed that the amino acids at positions 141 to 145 are crucial for binding vaccine-induced anti-HBs [6].

The rate of coexistence of anti-HBs and HBsAg in people infected with HBV varies depending on the disease's chronicity and severity [7-9]. This coexistence rate of anti-HBs and HBsAg was reported as 2.5% in people presenting for health-checkup. Remarkably, the anti-HBs coexistence rate was exceptionally high, up to 30%, especially in patients with active CHB [8]. Moreover, higher detection rates of anti-HBs coexistence were present in people with S region mutations or hepatocellular carcinoma (HCC) [7, 10]. In this study, we aimed to describe the coexistence rate of HBsAg and anti-HBs, examine any related factors, and investigate the relationship between anti-HBs coexistence and HCC in Vietnamese CHB patients.

Methods

Study design

This investigation recruited 521 outpatients with chronic HBV infection, who were followed up at the Liver Clinic of the University Medical Center (UMC) at Ho Chi Minh (HCM) City from April 2014 to December 2020. The enrolment criteria included patients ≥16 years of age, with a positive HBsAg presenting for more than 6 months, had HBV-DNA ≥5 log copies/mL (or ≥3 log copies/mL if HBeAg negative), had never been treated or discontinued nucleot(s)ides for at least 6 months before collecting samples, whether they had been diagnosed or presented with HCC or not [11].

Data collection, variables, and measurements

The data on quantitative HBsAg, HBV-DNA, HBV genotype, HCC, and cirrhotic status were extracted from the electronic database of UMC. Additional anti-HBs tests were done using the stored serum samples. The coexistence of HBsAg and anti-HBs status was defined in this study for patients who had positive HBsAg (>0.05 IU/mL, using the

quantitative by electrochemical immunochemistry assay, Elecsys HBsAg II Quant reagent, Roche, at the UMC lab) and of positive anti-HBs (≥10 mIU/mL, using the quantitative fluorescence electrochemical immunoassay, Cobas reagent, Roche on Elecsys and Cobas e systems at the Medical Diagnostic Center at Ho Chi Minh City). The HBV-DNA levels were measured by the in-house real-time PCR at the UMC laboratory (Limit of detection (LOD)=300 copies/mL).

Genotyping of HBV was determined based on sequences of gene S under Sanger sequencing, compared to the referenced sequences specified by genotypes B and C.

HCC was defined in those with tumor lesions observed on abdominal ultrasound and was confirmed by an abdominal CT scan that detected focal lesions with enhancement pattern as early arterial enhancement and early "washout" with serum alpha-fetoprotein (AFP) > 20 ng/mL [12].

S mutation was analyzed by Sanger sequencing

The mutations on the S (small S) gene analysis were done selectively on 177 non-HCC patients. Primer sequences for amplifying S region were described in our previous study [13]. Briefly, HBV DNA were extracted from serum using GeneJet^{TM} Viral DNA and RNA Purification kit (Thermo Fisher Scientific, Waltham, MA, USA). The small S region was amplified by PCR with Takara Taq^{TM} HotStart Polymerase (Takara Bio, Shiga, and Japan) and its PCR product was checked on 1.5% agarose gel. PCR products were purified enzymatically using ExoSAP-IT^{TM} PCR Product Cleanup Reagent (Thermo Scientific, Waltham, MA) to the removal of excess primers and dNTPs before Sanger sequencing using a BigDye Terminator v3.1 Kit and ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). PCR fragments were sequenced and

analyzed in both directions. Sequences were aligned by using the CLC Main Workbench software (Qiagen, Germany). HBV genotypes were determined based on the conserved nature of nucleotide sequences in regions of the S gene. Reference sequences, including genotype B (Genbank_AB073846) and genotype C (GenBank_X04615), were used for identifying mutations on S gene.

Statistical analysis

The statistical analysis and data visualizations were done using IBM SPSS software, version 25.0. Summary statistics were done using substantial numbers of events accompanied by percentages. Comparing different groups was done using the Mann-Whitney U test to compare the medians, interquartile ranges, and ranges of non-normally distributed continuous variables. Categorical variables were compared using Fisher's Exact test or Chi-Square test. Multivariable logistic regression was conducted to investigate the factors associated with the coexistence of anti-HBs. Also, the coexistence of anti-HBs and their correlation with HCC were assessed using univariable and multivariable analyses. Statistically significant difference was defined when the p-value was <0.05.

Ethical statement

This study was conducted per the principles of the Declaration of Helsinki and was approved by the ethical board of the University of Medicine and Pharmacy at Ho Chi Minh City (ID: 136/DHYD-HD) on April 17th, 2014. All stored serum samples and variables used for the analysis in this study were shared from a former investigation.

Results

Population characteristics

A total of 521 patients diagnosed as chronic HBV patients were included in this study. Of those, 350 patients were male (62.7%). The median age was 41 (IQR 32-52) years. 89 patients (17.1%) had hepatic fibrosis (≥F3), and 51 patients (9.8%) had HCC (Table 1). Furthermore, half the patients (51.1%) had negative HBeAg. HBV genotype C or B&C occupied 28.6%. The median level of HBV-DNA was 6.57 log cps/mL, and the median HBsAg was 3.4 log IU/mL (Table 1).

The rate of HBsAg and anti-HBs coexistence

51 patients had coexistence HBsAg and anti-HBs, which accounted for 9.8% of the study population (Table 1). There were no differences in rates of coexistence of anti-HBs according to HBeAg, sex, levels of HBV-DNA groups, and liver fibrosis status. However, significantly higher rates of coexistence anti-HBs were observed in patients infected with genotype C (compared to genotype B, 17.9% vs. 7.4%, p=0.001) and in those with HCC (compared to the non-HCC group, 19.6% vs. 8.7%, p=0.013) (Table 2). The median (IQR) levels of HBsAg in the group with coexistence was 3.2 (95% CI: 2.6-3.8) log IU/mL, slightly lower than that in those without coexistence anti-HBs (3.4, 95% CI: 2.96-4.2) log IU/mL, with borderline p-value (0.052) (Table 2).

Using correlation visualization, the values of HBsAg were widely distributed with a density from 2.5 to 5 log IU/mL. The anti-HBs values were distributed in two manners. One group with low values of anti-HBs (<100 IU/mL) but a wide variety of HBsAg values. The other group with a high or very high anti-HBs value (>100 IU/mL, included 4 cases of >500 IU/mL) in accordance with high HBsAg values (>3 log IU/mL) (Figure 1).

Factors associated with the coexistence status in our study population

According to univariable analysis, three characteristics found to be related to the coexistence of HBsAg and anti-HBs status with $p<0.1$ were genotype C ($p<0.001$), low HBsAg level (<3 log IU/mL) ($p=0.068$), and the presence of HCC ($p=0.013$) (Table 2). The multivariable analysis that included the three above factors was processed on 409 patients and identified genotype C and low HBsAg level (<3 log IU/mL) as the two factors associated with the anti-HBs coexistence in CHB patients (OR=3.93, 95% CI 2.1-7.38, $p<0.001$, and OR=1.03, 95% CI 1.07-3.88, $p=0.031$, respectively) (Table 3).

Mutation characteristics on the S gene in non-HCC groups with and without anti-HBs

The sequences of HBV S gene of 177 non-HCC patients were selectively analyzed to investigate mutations. Among those, 42 patients had anti-HBs coexistence and the remaining 135 patients had anti-HBs negative. Table 4 presented higher rates of anti-HBs coexistence in the group of patients with at least one point mutation on the major hydrophilic region (MHR) compared to the MHR wild-type group (29% vs. 15.7%, $p=0.043$); and predominately higher in the group with at least one point mutation on the "a" determinant sequence compared to the group without any point mutation in the same region (34.3% vs. 16.8%, $p=0.008$). Related to individual point mutation characteristics, the rate of anti-HBs coexistence was also higher in the group of patients with at least one of these S point mutations: L42P/R (75% vs. 22.5%, $p=0.042$), T/V47E/K/A (43.8% vs. 21.7%, $p=0.048$), and T/I126N/I/S/A (35% vs. 20.4%, $p=0.057$) (Table 4).

People at risk of developing HCC

Table 5 presented the univariable and multivariable analysis that included five factors associated with HCC (such as sex, age group, liver fibrosis, anti-HBs coexistence, and HBV genotype) to investigate the correlation between anti-HBs coexistence and HCC. There were four factors that have an increased risk of HCC: being male (OR=2.53, 95% CI: 1.2-5.36, p=0.015), being 40 years or older (OR=3.32, 95% CI: 1.56-7.06, p=0.002), presenting with liver fibrosis (OR=2.78, 95% CI: 1.44-5.38, p=0.002), and having anti-HBs (OR=2.94, 95% CI: 1.56-7.06, p=0.009).

Discussion

Our study population included 521 CHB patients, with 61.6% infected with HBV genotype B, 51.1% negative with HBeAg and the median (IQR) of HBV DNA = 6.57 (5.0-8.0) log copies/mL, were similar to the general HBV populations in Vietnam. However, at such a tertiary care level hospital like UMC in HCM city, where many patients with complicated diseases tend to present or be referred to, therefore more patients with cirrhosis (17.1% of them were at ≥F3) and hepatocellular carcinoma (9.8% HCC) had been enrolled in this study. All patients with stored serum got the anti-HBs test, not at the same time as the HBsAg test, confirming actual coexistence with antiHBs >10 mIU/mL by specificity electrochemical immunochemistry test. The overall detection rate of anti-HBs coexistence on 521 patients who were HBV diagnosed in the study was 9.8%, and in our non-HCC group should be 8.7% (41/470 cases). Although the study had included HCC cases (9.8%, 51/521 patients), our overall anti-HBs coexistence detection rate was much higher than that of other published papers such as that from Colson P et al. (2.8%, n=459) [5], Xiang Y et al. (0.3%, n=24,856) [14], Lee BS et al. (2.9%, n=290,212 on Korean HBV on health check-ups detected population)

[15] and Pancher M et al. (5%, n=2578) [16]. Especially, higher rates of anti-HBs coexistence were reported by Jang JS et al. (6.4%, n=755) in a study whose sample was 8.9% HCC patients [7] comparingly to ours (9.8%). Regarding the cohort study of Seo SI et al. (2014) on 1042 non-HCC CHB patients, 7.0% of them detected HBsAg and anti-HBs coexistence after a median follow-up time of 4.3 years (from 1.0–22 years) [10]. The reported paper from Seo SI et al. proved that coexistence was an actual and progressing status in CHB populations. Hayashi et al. found chronic HBV carriers with anti-HBs and HBsAg coexistence had HBsAg and anti-HBs of different serotypes [17]. Chronic Hepatitis B patients could be reinfected with second subtypes [18]. And in patients with mixed infection of different HBV serotypes, anti-HBs composed incomplete cross-immunity [19]. Many different patterns of anti-HBs are produced in chronic Hepatitis B patients to immunity responses, and the patterns of surface antibodies may reflect the prediction for surface antigen clearance and the timing of clearance HBsAg [20], [21].

Regarding the distribution of anti-HBs by HBsAg values from 51 cases with anti-HBs coexistence from our study, we found only 10 patients with exceptionally high values of both HBsAg and anti-HBs (real anti-HBs coexistence). Most HBsAg values were accompanied by low levels of anti-HBs (Figure 1). This low anti-HBs might relate to the processing of anti-HBs during HBsAg seroconversion (false positive anti-HBs), the non-dominant and suppressed serotype in multiple serotypes infection; or the anti-HBs that was linked to the HBsAg mutated strain among the wild and mutated mixture HBV population. Especially, these low levels HBsAg might originate from the integrated HBV viral genome on the host DNA [20], from the truncated cccDNA in the hepatic

nucleus, or from the HBsAg-antiHBs immune complexes [22]. A cohort observation with a similar design as Seo SI et al. study could better differentiate the real anti-HBs coexistence or the progression of HBsAg seroconversion in this low HBsAg and anti-HBs coexistence group [10].

Among HBeAg positive group, our detected rate of anti-HBs coexistence (10.6%) was far higher than the rate of 4.9% that was reported by Zhang JM et al. on 411 HBeAg positive and non-advanced liver injury CHB patients [23]. Regarding, the anti-HBs coexistence rates were not different among the under and the over-30 age groups (12.2% vs. 10.3%) (Table 2). This similarity of anti-HBs coexistence rates between these age groups on the HBeAg positive subpopulation disclosed that the coexistence status might arise early during chronic infection. It might be inconsiderably accumulative after HBeAg seroconversion. The distribution of coexistence was not different according to gender, HBV DNA, and HBsAg level. These findings had not given evidence of whether the coexistence status happened during the progression of HBV infection**.** These 51 patients had active HBV infections with a median viral load of 6.71 log cps/mL compared to patients without coexistence of 6.54 log cps/mL with no significant difference.

As for the association between genotype C and anti-HBs coexistence in our study (OR= 3.93 (95% CI 2.1-7.38, $p<0.001$) (Table 2), the different sequence of the S gene that was used to define the genotype in the study revealed indirect evidence for the genetic cause of HBsAg and anti-HBs coexistence. Basal core promoter mutation and mutations on the S gene have also been stated to explain the higher rates of HCC among genotype

C [24, 25]. The association of coexistence with genotype C needs to be considered for the higher genotype C HCC risk. Moreover, we also found the association of lower HBsAg levels with anti-HBs coexistence on multivariable analysis. This finding was similar to Liu J et al. [26].

In occult HBV infection patients whose HBV was reactivated by immuno-suppression therapy, viral replication would produce a large amount of HBsAg in a short time, leading to the coexistence situation [27]. Kim HS et al. reported 237 distinct (including 25 novels) mutations on the MHR region in HBV-infected East- and Southeast Asian patients. The Elecsys® HBsAg II Qualitative assay can reliably detect HBV-positive samples with a sensitivity of 100% (95% CI: 99.59–100.0) for all 898 samples with and without mutations [28]. Mutations in the "a" determinant region (vaccine escaping mutants) caused a decrease in the affinity for anti-HBs, and the G145R was discovered as the most common immune-escape mutation .

Additionally, the cut-off value of HBsAg <3 log IU /mL is advocated and applied for the HBV inactive state. In this study, the anti-HBs coexistence status was found prominent among our low HBsAg (<3 log IU/mL) group (14.7% vs... 9%). Therefore, in the region with a notable rate of anti-HBs coexistence, the above advocacy for mother to child prevention based on HBsAg level of <3 log IU/mL might be cautious, especially in these abnormally low HBsAg population.

Related to HBV progression and patient management, the truncated and low HBsAg strain has been considered related to HCC progression. More studies are needed to recognize possible abnormal low HBsAg for further sequencing diagnosis, and to define

the appropriate strategy on diagnosis, treatment, and morbidity prevention for those patients with unusually low levels of HBsAg. Related to this aim, there were no age groups, gender, or HBeAg that could help, but screening for anti-HBs coexistence and periodic HBV DNA quantification for the low HBsAg level might be of value, especially in personalized or precision medicine.

Related to the morbidity role of anti-HBs coexistence, Heijtink RA et al. (1982) (n=89) found lower anti-HBs coexistence rates in asymptomatic HBV carriers compared to the active CHB group (3/23 vs. 20/40, included five cirrhosis patients). They had concluded about the pathological relation between anti-HBs coexistence status and advance of liver disease [24]. Although liver cirrhosis was not declared in our study, our analysis also indicated a significant prevalence of the coexistence of anti-HBs in the HCC group (19.6% vs. 8.7%, p=0.013) compared to the non-HCC group (Table 2). These findings on the higher prevalence of anti-HBs and HBsAg coexistence in the advanced liver group and the HCC group revealed any possible pathogenic role of this anti-HBs coexistence.

Only a few studies related to the association of S mutations with the coexistence of anti-HBs and with HCC until now. In our study, we found high rates of cases that possessed point mutation(s) on the MHR (60.5%) and “a” determinant (39.5%) region, and the association of the mutations on the MHR and “a” determinant regions with antiHBs coexistence. Other studies had detected much lower rates than our study on the “a” determinant (2.4% vs. 9.5%, p=0.009, Lada O et al. (2006) [6] and 2.01% vs. 7.14%, p <0.001, Wang J et al. (2010) [29]; on the MHR (1.38% vs. 2.20%, p <0.001); Moreover, Ding F et al. reported common mutation as sI126S/T (40%) and other mutations as

sQ129R, sG130N, sF134I and sG145R on 15 anti-HBs positive genotype C CHB patients [30]. Differently, we found lower rates of sT/I126/N/I/S/A (22.6%) and did not find their association with coexistence. Specifically, we detected another two point mutations associated with anti-HBs coexistence as sL42P/R and sT/V47E/K/A.

The mutations on the MHR and "a" determinant regions that activate the immunological response were explained for the higher risk of advanced liver diseases and HCC in anti-HBs coexistence CHB patients [19, 29, 31]. This study found the association between anti-HBs coexistence and HCC (OR=2.55; 95% CI 1.19-5.47) and its higher OR for HCC (OR=2.94; 95% CI 1.31-6.62) were also proven on the multivariable analysis. Other widely accepted cofactors such as male, older than 40 years, and liver fibrosis have also been found in ours and other studies [7, 10]. The higher HCC rate in the group with the coexistence of anti-HBs had also been stated in other studies (22.9% (11/48) vs. 7.9% (56/707), $p = 0.002$, Jang JS et al. (2009) [7]. Anti-HBs coexistence and three remaining factors had also been proven by Seo SI et al. (2014) [10]. These related factors proved that chronic HBV infection in patients is associated with longer infection and that immunity-activated inflammation with or without advanced liver fibrosis leads to liver regeneration with neogenesis formation. More research would be needed to determine the role of HBsAg and anti-HBs coexistence in HCC occurrence, especially in patients with advanced liver diseases.

Conclusion

The coexistence of anti-HBs with HBsAg was at a higher rate in HBV genotype C and low HBsAg groups. Mutations on the MHR and "a" determinant regions (especially

sL42P/R, sT/V47E/K/A, and sT/I126N/I/S/A point mutations) were associated with the status of anti-HBs coexistence. We have also found an association between the coexistence status and HCC in this cross-sectional study. More studies need to be established to determine the pathological role(s) of anti-HBs coexistence and its relations with S gene mutations in chronic HBV infection.

Tables and figures legends

Table 1. Baseline characteristics and HBV markers characteristics of study population (n=521).

Table 2. The distribution of the coexistence status among population and viral characteristic groups (n=521).

Table 3. Factors associated with the coexistence of anti-HBs in multivariable analysis (n=409).

Table 4. Distribution of the mutations on the S region among the groups with anti-HBs coexistence (n=177).

Table 5. The coexistence of anti-HBs and factors correlation with HCC using univariable and multivariable analyses (n=521).

Figure 1. Levels of HBsAg and anti-HBs in CHB patients with coexistence of HBsAg and anti-HBs (n=51).

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Table 1. Baseline characteristics and HBV markers characteristics of study population (n=521).

Characteristics		**n (%)**
Gender (male)		350 (67.2)
Age group (years)	**<30**	98 (18.8)
	30-50	272 (52.2)
	>50	151 (29)
Hepatic fibrosis (≥F3) (yes)		89 (17.1)
Hepatocellular carcinoma (yes)		51 (9.8)
HBV Genotype	**B**	321 (61.6)
	B/C and C (grouped)	149 (28.6)
	Unspecified	51 (9.8)
HBeAg negative (yes)		266 (51.1)
HBV-DNA (log copies/mL)	**<5**	130 (25.0)
median (IQR) = 6.57 (5-8.0)	**5-8**	256 (49.1)
	>8	135 (25.9)
HBsAg (log IU/mL) (n=460)	**≤3**	136 (26.1)
median (IQR) = 3.4 (2.9-4.1)	**>3**	324 (62.2)
Anti-HBs >10 mIU/mL (yes)		51 (9.8)

Table 2. The distribution of the coexistence status among the population and viral characteristic groups (n=521).

Characteristics		**Coexistence, n (%)**		**p-value**[a]
		Yes (n=51)	**No (n=470)**	
Gender	**Male**	34 (9.7)	316 (90.3)	0.940
	Female	17 (9.9)	154 (90.1)	
Age group	**<30**	12 (12.2)	86 (87.8)	0.430
	30-50	28 (10.3)	244 (89.7)	
	>50	11 (7.3)	140 (92.7)	
HBeAg	**Positive**	27 (10.6)	228 (89.4)	0.550
	Negative	24 (9)	242 (91)	
Genotype (n=470)	**B**	24 (7.4)	301 (92.6)	0.001
	C	26 (17.9)	119 (82.1)	
HBV-DNA (log cps/mL)	**<5**	14 (10.8)	116 (89.2)	0.660
	≥5	37 (9.5)	354 (90.5)	
HBsAg (log IU/mL) (n=460)	**<3**	20 (14.7)	116 (85.3)	0.068
	≥3	29 (9)	(91)	
	Median (IQR)	3.2 (2.6-3.8)	3.4 (2.96-4.2)	0.052[b]
HCC	**Present**	10 (19.6)	41 (80.4)	0.013
	Absent	41 (8.7)	429 (91.3)	
Liver fibrosis ≥F3	**Present**	10 (9.6)	79 (16.8)	0.610
	Absent	41 (80.4)	391 (83.2)	

All percentages are calculated per row, [a] Chi square test, [b] Mann-Whitney U test

Table 3. Factors associated with the coexistence of anti-HBs in multivariable analysis (n=409).

Variables		**OR**	**95% CI**	**p-value**
Genotype	**B**	1		
	C	3.93	2.10-7.38	<0.001
HBsAg (log IU/mL)	**≥3**	1		
	<3	2.03	1.07-3.88	0.031

Table 4. Distribution of the mutations on the S region among the groups of anti-HBs coexistence (n=177)

Mutation		Sample (n=177)	Anti-HBs coexistence, n (%)		p-value[a]
			Yes (n=42)	No (n=135)	
By region (at least one point mutation)					
MHR	**Yes (>=1)**	107 (60.5)	31 (29)	76 (71)	0.043
	No	70 (39.5)	11 (15.7)	59 (84.3)	
The "a" determinant region	**Yes (>=1)**	70 (39.5)	24 (34.3)	46 (65.7)	0.008
	No	107 (60.5)	18 (16.8)	89 (83.2)	
Point-mutations					
L42P/R	**Yes**	4 (2.3)	3 (75)	1 (25)	0.042
	No	173 (97.7)	33 (22.5)	134 (77.5)	
T/V47E/K/A	**Yes**	16 (9)	7 (43.8)	9 (56.3)	0.048
	No	161 (91)	35 (21.7)	126 (78.3)	
T/I126N/I/S/A	**Yes**	40 (22.6)	14 (35)	26 (65)	0.057
	No	137 (77.4)	28 (20.4)	109 (79.6)	

All percentages are calculated per row, , [a] Chi square test

Table 5. The coexistence of anti-HBs and factors correlation with HCC in univariable and multivariable analyses (n=521).

Variables		Univariable			Multivariable		
		OR	**95% CI**	**p**	**OR**	**95% CI**	**p**
Sex	**female**	1		0.038	1		0.015
	male	2.14	1.04-4.38		2.53	1.2-5.36	
Age group	**<40**	1		<0.001	1		0.002
	≥40	3.96	1.94-8.1		3.32	1.56-7.06	
Liver fibrosis	**no**	1		<0.001	1		0.002
	yes	3.75	2.02-6.95		2.78	1.44-5.38	
Anti HBs	**no**	1		0.016	1		0.009
coexistence	**yes**	2.55	1.19-5.47		2.94	1.31-6.62	
HBeAg	**positive**	1		0.146			
	negative	0.65	0.36-1.16				
Genotype	**B**	1		0.004			
	C	2.42	1.34-4.37