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CLINICAL STUDY

Distribution and relevance of hepatitis B genotypes in the general population of Slovakia

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ABSTRACT
AIMS: The aim of the presented study was to determine the distribution of HBV genotypes and their influence on selected parameters in patients in eastern Slovakia.
METHODS: The study includes 202 patients with confirmed chronic HBV infection or hepatitis. For each patient, basic demographic data, and serum samples were collected. The degree of liver fibrosis was determined by transient elastography. The obtained data were evaluated statistically.
RESULTS: Out of a total of 202 patients, 96.0 % of the patients were from the EU region and 27 patients (13.4 %) self-identified as Roma ethnic group. The most common genotype among our patients was genotype A (n = 104; 51.5 %), followed by genotype D (n = 76; 37.6 %) and A/D (n = 13; 6.4 %). In patients from the EU region, genotypes A and D predominated statistically significantly ($p < 0.0001$). Due to a low number of patients with other genotypes, in the subsequent analysis, we only compared patients with HBV genotypes A or D. Patients with genotypes D and A/D significantly more often mention tattoos as a possible risk factor for disease transmission compared to patients with genotype A ($p = 0.043$). Subsequently, we divided patients into two groups – treated and untreated. The level of qHBsAg was significantly higher in untreated patients with genotypes A ($p < 0.0001$). The influence of HBV genotypes on other laboratory parameters was not confirmed in our study.
CONCLUSION: This is the first HBV genotypes study from Slovakia. We suggest that HBV genotypes may play a role in the virus-host relationship *(Tab. 5, Fig. 1, Ref. 27)*. Text in PDF *www.elis.sk*
KEY WORDS: chronic hepatitis B, genotypes, hepatitis B virus, prognostic factors, distribution.

Introduction

Chronic hepatitis B (CHB) is a long-lasting and potentially life-threatening liver infection caused by the hepatitis B virus (HBV). Due to its global distribution and related health problems, chronic hepatitis B is considered a major health problem. The main route of transmission is through contact with infected blood or other body fluid. World Health Organization (WHO) estimates that 296 million people were living with chronic hepatitis B in 2019, and approximately 820 000 individuals died due to HBV-related diseases this year. The highest prevalence of HBV infection is in the WHO Western Pacific Region and the WHO African Region (1, 2). The natural history of CHB is a dynamic process and can be divided into five phases: HBeAg-positive chronic infection, HBeAg-positive chronic hepatitis, HBeAg-negative chronic infection (inactive carrier), HBeAg-negative chronic hepatitis and HBsAg-negative phase (3). The prognosis of HBV infection depends on several factors such as access to health care, age of the patient, genotype of the virus, and coinfection (4).

HBV is classified into at least 10 HBV genotypes. These genotypes are differentiated by the sequence variations by a genome-wide sequence divergence of more than 8 %. They are named by capital letters A to J and they differ in geographic, ethnic distribution, and evolutionary rate. Additionally, multiple subgenotypes are identified (5). Genotype A is mainly prevalent in Africa, Europe, India, and North America and it has seven subgenotypes. Genotype B has nine subgenotypes and with genotypes C is the most common in the Asia-Pacific region. Genotype C is considered to be the oldest HBV genotype and it has the highest number of subgenotypes (16) (6). Genotype D is the most widespread in the Mediterranean region, Africa, Europe, and North America, even though it has a worldwide distribution. Nine subgenotypes D have

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been identified to date. Genotype E has been reported exclusively from West and Central Africa and Saudi Arabia and it has a very low degree of genetic diversity. Genotype F is classified into four subgenotypes and it is prevalent in Central and South America. Genotype G can be found in France, Germany, and North and Central America and it shows low genome diversity as well as genotype E. Genotype H is distributed in Central America and France and it is closely related to genotype F. Genotype I has been identified in Laos, Vietnam and the last genotype J is exclusive in Japan (1, 5, 6, 7, 8, 9).

HBV genotypes seem to be an important prognostic factor. Different genotypes may have varying responses to antiviral therapy and can influence the progression of liver disease, including the development of drug resistance. The higher intracellular expression of HBV DNA is typical for genotypes C and D compared to genotypes B and A (7). Progression to chronic infection occurs more commonly with genotypes A and D. Higher rates of spontaneous HBeAg seroconversion are seen in infection caused by genotypes A and B. The genotypes and subgenotypes A1, C, and D are associated with a higher risk of developing end-stage liver disease and HCC. Additionally, in individuals infected with genotype C, there is a higher chance of developing HCC in older age, whereby genotype B is associated with HCC that occurs at a younger age. In more detail, genotypes A1, C, B2-B5, and H appear to be connected to serious complications than genotypes A2, B1, and B6. Better response to antiviral treatment has been observed in genotypes A and B than in infection caused by genotypes C and D (7, 8, 10, 11, 12).

Materials and methods

Population study

The presented prospective observational study included individuals with a diagnosis of chronic HBV infection or chronic hepatitis B. Patients were recruited from June 2019 to June 2022. Two hundred and two HBV-infected patients were enrolled in this study. Patients were included if aged 18 years or older, diagnosed with chronic HBV carriage (defined as the presence of HBsAg for more than 6 months), and if agreed to participate in the study. Sera were collected from patients attending two hepatology outpatient centers at the University Hospital of Louis Pasteur in Kosice, Slovakia. These two centers cover the whole eastern Slovakia region with 1.8 million inhabitants. During the medical visits, standardized reporting forms were filled in. Selected demographical (sex, age, geographical origin, Roma ethnicity, height, weight, educational attainment, and employment status), epidemiological (history of intravenous drug use, tattoo, piercing, imprisonment), virological (quantitative hepatitis B surface antigen (qHBsAg), hepatitis B e antigen (HBeAg), HBV DNA, HBV genotypes), hematological (thrombocytes, neutrophil/lymphocyte (NE/LY) ratio, INR), biochemical (aspartate aminotransferase (AST) activity, alanine aminotransferase (ALT) activity, albumin, bilirubin, interleukin 6 (IL-6), high-sensitivity C-reactive protein (hs-CRP), alpha-fetoprotein (AFP), beta2-microglobulin, cholesterol), histological (severity of liver disease) data were collected at the time of sample collection. Written informed consent was obtained from all participating individuals, and the study was approved by the Ethics Committee of the University Hospital of Louis Pasteur, Kosice, Slovakia, No. 2019/EK/4022 from 25 April 2019.

HBV genotyping

HBV DNA was isolated from 400 µl of serum, using the QIAamp DNA Mini kit (QIAGEN GmbH, Hilden, Germany) in accordance with the manufacturer`s protocol and dissolved in 40 µl of elution buffer. In the case of samples with known or suspected low concentrations of viral DNA, 600 µl of serum was used for isolation, with a final elution volume of 15 µl. Samples were stored at −20 °C until PCR. HBV amplification and subsequent analyses were conducted according to the protocol described by Logoida et al. (2022), using primers targeting the surface genomic region (13). In the case of samples with known or suspected low concentrations of viral DNA, an additional 10 cycles were added to both PCR (direct and nested). HBV genotyping was done using 10 µl of extracted DNA and the commercial kit AmpliSens HBV-genotype-FRT (AmpliSens, Federal Budget Institute of Science "Central Research Institute for Epidemiology", Moscow, Russia)

Tab. 1. Characteristics of study participants by HBeAg status.

	Chronic HBV infection (n=113)	Chronic hepatitis B (n=89)	Total (n=202)
Sex			
male	65 (57.5 %)	57 (64.0 %)	122 (60.4 %)
female	**48 (42.5 %)**	**32 (36.0 %)**	**80 (39.6 %)**
HBeAg status			
HBeAg-positive	**0 (0 %)**	**11 (12.4 %)**	**11 (5.4 %)**
HBeAg-negative	113 (100 %)	78 (87.6 %)	191 (94.6 %)
Fibrosis			
mild (F0-F1)	86 (76.1 %)	51 (57.3 %)	137 (67.8 %)
moderate (F2-F3)	**14 (12.4 %)**	**21 (23.6 %)**	**35 (16.9 %)**
cirrhosis of liver	0 (0 %)	14 (15.7 %)	14 (7.4 %)
unknown	**13 (11.5 %)**	3 (3.4 %)	16 (7.9 %)
Geographical origin			
EU region	**108 (95.6 %)**	**86 (96.6 %)**	**194 (96.0 %)**
outside EU region	5 (4.4 %)	3 (3.4 %)	8 (4.0 %)
Roma ethnic group	**16 (14.2 %)**	**11 (12.4 %)**	**27 (13.4 %)**
Employment status			
employed	**92 (81.4 %)**	48 (54.0 %)	140 (69.3 %)
unemployed	16 (14.2 %)	36 (40.4 %)	52 (25.7 %)
unknown status	**5 (4.4 %)**	**5 (5.6 %)**	**10 (5.0 %)**
Educational attainment			
basic	**12 (10.6 %)**	**22 (24.7 %)**	**34 (16.8 %)**
secondary	63 (55.8 %)	49 (55.1 %)	112 (55.4 %)
university	33 (29.2 %)	17 (19.1 %)	50 (24.8 %)
unknown attainment	5 (4.4 %)	1 (1.1 %)	6 (3.0 %)

according to the manufacturer`s protocol. The AmpliSens HBV-genotype-FRT PCR kit is a nucleic acid amplification test for qualitative detection and differentiation of HBV genotypes A, B, C, and D. Amplification was performed on a LightCycler 480 Real-Time PCR System (ROCHE Diagnostics, Mannheim, Germany).

Assessment of liver fibrosis

The fibrosis stage was assessed by transient elastography (Fibroscan). Mild fibrosis (F0-F1) was defined as a FibroScan score < 7.2 kPa, and moderate fibrosis was defined as F2 (7.2–9.4 kPa) or F3 (9.5–12.2 kPa). Liver cirrhosis was defined as a FibroScan score > 12.2 kPa.

Statistics

Data are presented as absolute and relative counts in the case of categorical variables. Interval variables are presented as mean and standard deviation in the case of normal distribution or median and interquartile range in the case of not normal distribution. Variables with no normal distribution were log-transformed before analysis. The significance of differences was tested by T-test or Kruskall–Wallis test in case of continuous variables and the Chi-squared or Fisher exact test in case of categorical variables while respecting the tests´ assumptions. p values of less than 0.05 were considered significant.

Results

Characteristic of the study population at referral

A total of 202 patients were enrolled in the study. Eighty patients were females (39.6 %) and 122 males (60.4 %). The median age at referral was 47 years (range 21–82 years). No significant age difference was found between males and females (46 (29–78) vs 47 (21–82) years, respectively). The majority of patients were from the EU region (n = 194, 96.0 %) and 27 patients (13.4 %) self-identified as Roma ethnic group. Five patients were of Vietnamese origin (2.5 %), and another 3 (1.5 %) were from Nigeria, Ukraine, and Azerbaijan. Fifty-two patients (25.7 %) were unemployed at the time of inclusion into the study. Information on educational attainment was available for 196 patients and information about employment status was known in 192 patients. Most of the patients had secondary education (n = 112; 55.5 %). There were 50 (24.8 %) patients with university education and 34 (16.8 %) with basic education. One hundred and forty patients were employed (69.3 %). We have collected information about the most common risk factors for transmission of HBV. Four patients (1.9 %) admitted intravenous drug use in the past, 32 patients (19.9 %) were tattooed, and 6 patients (2.9 %) were in prison. In the majority of patients (n = 160; 79.2 %) the source of infection stayed unknown. The median duration of known HBV infection was 147 months (min <1 month and maximum 585 months). The length of infection was evaluated based on anamnestic data from the first confirmation of HBV diagnosis to the time of collecting data.

One hundred and thirteen patients (55.9 %) were diagnosed with chronic HBeAg-negative infection (asymptomatic carriers), the rest of them, 89 (44.1 %) patients were diagnosed with chronic HBV hepatitis. Eleven patients were HBeAg-positive (5.4 %), and 78 patients (38.7 %) were HBeAg-negative. Sixty-two patients (30.7 %) were on antiviral treatment at the time of inclusion.

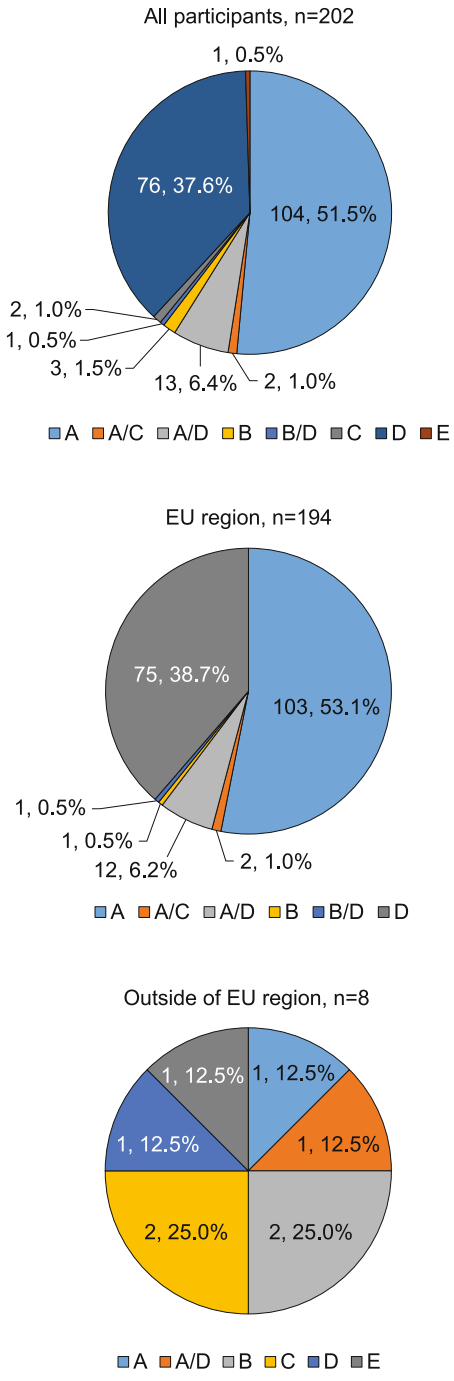

Fig. 1. Distribution of HBV genotypes in study participants.

Transient elastography results were obtained in 186 individuals. One hundred thirty-seven patients (67.8 %) had no or mild fibrosis (F0–F1), moderate fibrosis (F2 and F3) was diagnosed in 35 patients (16.9 %) and 14 patients had liver cirrhosis (7.4 %).

Tab. 2. Demographic, epidemiological, and clinical characteristics of study participants and subgroup comparison by HBV genotypes.

	Genotype A		Genotype D		Genotype A/D		Other genotypes		p
	Count	% or SD	Count	% or SD	Count	% or SD	Count	% or SD	
female	43	41.3	30	39.5	6	46.2	1	11.1	0.331
male	61	58.7	46	60.5	7	53.8	8	88.9	
EU region	103	99.0	75	98.7	12	92.3	4	44.4	<0.0001
outside EU	1	1.0	1	1.3	1	7.7	5	55.6	
Roma ethnicity	13	12.5	10	13.2	4	30.8	0	0	0.182
unemployed	24	23.5	23	31.9	5	38.5	0	0	0.124
basic ed.	17	16.7	13	18.1	4	30.8	0	0	0.414
secondary ed.	56	54.9	45	62.5	5	38.5	6	66.7	
university ed.	29	28.4	14	19.4	4	30.8	3	33.3	
IVDU	1	1.0	2	2.7	1	7.7	0	0	0.387
tattoo	10	9.8	17	23.3	3	23.1	2	22.2	0.091
prison	2	2.0	3	4.1	1	7.7	0	0	0.589
length of infection (months)	202.7	136.2	176.6	202.4	93.4	116.3	154.8	171.3	0.01

IVDU – intravenous drug use, ed. – education

Tab. 3. Comparison of selected laboratory parameters in untreated and treated patients and HBV genotypes A and D.

	Untreated patients						
	Genotype A			Genotype D			p
	Mean	SD	Count	Mean	SD	Count	
PLT (10^9/L)	244	50	67	226	53	59	0.056
NE/LY ratio	1.75	.71	67	1.80	.70	57	0.696
Alb (g/L)	44.4	3.1	68	43.4	3.5	59	0.103
Bil T (µmol/L)	14.3	7.6	68	12.7	6.4	59	0.229
ALT (µkat/L)	.79	1.51	68	.63	.44	59	0.454
AST (µkat/L)	.61	.79	68	5.19	1.21	59	0.099
Chol (mmol/L)	5.52	1.02	68	5.19	1.21	59	0.099
	Treated patients						
	Genotype A			Genotype D			p
	Mean	SD	Count	Mean	SD	Count	
PLT (10^9/L)	225	73	36	232	78	17	0.741
NE/LY ratio	2.05	.83	36	1.96	1.25	17	0.756
Alb (g/L)	43.9	3.8	36	44.6	4.3	17	0.592
Bil T (µmol/L)	11.8	5.7	36	15.0	6.6	17	0.076
ALT (µkat/L)	.57	.25	36	2.20	3.55	17	0.077
AST (µkat/L)	.49	.20	36	1.16	1.60	17	0.108
Chol (mmol/L)	4.81	1.38	36	4.69	.68	17	0.727

PLT – platelets, NE/LY index, Alb – albumin, Bil T – total bilirubin, ALT – alanine aminotransferase, AST – aspartate aminotransferase, Chol - cholesterol

Only one patient (0.9 %) with HBeAg-positive chronic HBV hepatitis was diagnosed with liver cirrhosis. The rest of the patients (13; 7.0 %) with LC were HBeAg-negative. The data are summarized in Table 1.

HBV genotypes results

HBV genotyping was based on the results of the amplification and sequencing of the surface genomic region. By PCR amplification (direct or nested), it was possible to identify genotypes in all 202 samples, even in samples with low concentrations of viral DNA, after modifying the extraction method. On the other hand, genotyping using Real-Time PCR was not successful in all samples. Even after increasing the original sample volume and decreasing the final elution volume, amplification curves were absent in samples with low amounts of template DNA. The most frequent genotypes based on sequence analysis were A (n = 104; 51.5 %), followed by D (n = 76; 37.6 %) and A/D (n = 13; 6.4 %). We divided patients based on geographical origin into two groups: the EU region and outside of the EU region. In patients of EU origin, HBV genotypes A (n = 103; 53.1 %) and D (n = 75; 38.7 %) were the most prevalent, compared to patients outside the EU region, where the most prevalent genotypes were B (n = 2; 25.0 %) and C (n = 2; 25.0 %). This difference was statistically significant ($p < 0.0001$). Genotypes in all groups are summarized in Figure 1.

We compare HBV genotypes and their influence on selected demographical, epidemiological, and clinical parameters. Patients with HBV genotypes A, D, and A/D had a longer length of infection in comparison to patients with other genotypes ($p = 0.01$). Patients with HBV genotypes A, D, and A/D more often reported having tattoos in the past than patients with other HBV genotypes, but this difference was not statistically significant. Similarly, the comparison of other parameters, such as Roma ethnicity, employment status, educational level, and imprisonment, was not statistically significant as we can see in Table 2.

Comparison HBV genotypes A and D

In further analysis, we compared only patients with genotypes A and D because of the low number of patients with other genotypes. In the analysis of demographic data, patients with genotype D (n = 17; 23.3 %) or A/D (n = 3; 23.1 %) reported having a tat-

Tab. 4. Comparison of selected laboratory parameters in untreated and treated patients and HBV genotypes A and D.

	Untreated patients						
	Genotype A			Genotype D			p
	Median	IQR	Count	Median	IQR	Count	
INR	.99	0.11	66	.98	0.09	58	0.393
hs-CRP (mg/L)	1.5	2.21	68	1.7	2.76	58	0.238
IL-6 (pg/mL)	1.6	1.75	68	2.2	2.43	59	0.763
AFP (μg/L)	2.71	2.23	68	2.91	2.09	59	0.112
□2 micro (mg/L)	1.83	0.45	68	1.88	0.58	59	0.225
HBV DNA (IU/mL)	3 575	17 462	68	3 398	19 740	59	0.828
qHBsAg (IU/mL)	8 603	21 081	54	2 179	4 465	37	<0.0001
	Treated patients						
	Genotype A			Genotype D			p
	Median	IQR	Count	Median	IQR	Count	
INR	1.02	0.15	36	1.01	0.09	17	0.393
hs-CRP (mg/L)	1.2	2.07	33	1.4	1.76	17	0.238
IL-6 (pg/mL)	1.5	1.45	33	1.8	2.16	16	0.763
AFP (μg/L)	2.77	1.54	33	2.83	3.26	17	0.112
□2 micro (mg/L)	2.07	0.8	33	2.205	0.99	16	0.255
HBV DNA (IU/mL)	13	57	36	22	405	17	0.828
qHBsAg (IU/mL)	6 013	16 088	35	7 633	22 756	14	0.479

INR – international normalized ratio, hs-CRP – high-sensitivity C-reactive protein, IL-6 – interleukin 6, AFP – alpha-fetoprotein, beta2 micro – beta2-microglobulin, qHBsAg – quantitative HBsAg

Tab. 5. Comparison of liver fibrosis in untreated and treated patients and HBV genotypes A and D.

	Untreated patients						
Fibrosis	Genotype A		Genotype D		Total		p
	Count	%	Count	%	Count	%	
F0-F2	52	85.2	48	88.9	100	87.0	
F3-F4	9	14.8	6	11.1	15	13.0	0.563
Total	61	100	54	100	115	100	
	Treated patients						
Fibrosis	Genotype A		Genotype D		Total		p
	Count	%	Count	%	Count	%	
F0-F2	30	85.7	11	68.8	41	80.4	
F3-F4	5	14.3	5	31.3	10	19.6	0.157
Total	35	100	16	100	51	100	

too in the past significantly more often than patients infected with genotype A (10; 9.8 %), p = 0.043. A longer duration of known infection was observed in patients with genotype A (189.5 months), while patients with genotype A/D had the shortest duration of known infection (64.3 months), p < 0.003.

In the whole population study, there were only 11 patients with HBeAg positivity, in the subgroups of patients with HBV genotypes A and D there were 9 patients with HBeAg positivity. HBeAg positivity was significantly more frequent in patients infected with genotype D (n = 7; 9.2 %) than in those infected with genotype A (n = 2; 1.9 %), p = 0.037.

In further analysis of laboratory findings, we compared the influence of HBV genotypes A and D on monitored laboratory parameters. Individual data were analyzed in two groups: treated with antiviral agents and untreated. qHBsAg level was the only statistically significant marker. Untreated patients infected with HBV genotype A had higher levels of qHBsAg (p < 0.0001). A similar finding was not observed in the group of treated patients. Comparisons of another laboratory finding were not statistically significant. A closer overview of laboratory parameters is shown in Tables 3 and 4.

In the assessment of the level of liver fibrosis, we have also divided patients into two groups: treated with antiviral agents and untreated. In both groups, a statistically significant difference in the influence of HBV genotype on the level of liver fibrosis was not found. However, in the group of treated patients, there was an increase in the level of liver fibrosis in patients infected with genotype D compared to patients with genotype A (31.3 % vs 14.3 %) as Table 5 shows.

Discussion

The prevalence of HBV in the general population in Europe ranges from 0.1 % to 7 %, depending on the country. Low HBV endemicity is reported in the countries such as Belgium, Italy, Germany, the Czech Republic, and the Slovak Republic (SR). The exact prevalence of HBsAg positivity in SR is not known, no prevalence studies have been published. The last modeled estimated prevalence of HBsAg positivity in SR is 0.81 %. Based on data from the Statistical Office of the Slovak Republic, as of December 31, 2022, the Slovak Republic had 5 428 792 inhabitants. This represents approx. 44 000 people living with chronic HBV infection (14, 15). In our study we present the first unique data about HBV genotypes in the Slovak population. In view of the given data, we consider our study to be representative.

HBV genotypes are geographically distributed among the 5 continents. In our study, the most prevalent genotypes in the EU region were genotypes A and D (n = 178, 91.8 %). These findings are consistent with data from multiple studies. Genotype A is mainly prevalent in northwestern Europe and south-eastern Africa. Genotype D is predominant in the Mediterranean basin, other parts of Europe, and some parts of Asia (4, 5). The specific global distribution of HBV genotypes is also associated with different transmission modes. In highly endemic areas, such as some Asian countries, HBV genotypes B and C are the most prevalent. For these two genotypes, the perinatal or vertical (from mother to child) mode of transmission is the most typical (16). Horizontal mode of transmission is more frequently observed in HBV genotypes

A and D (17). In our analysis patients with genotypes A/D and D reported having tattoos more frequently than patients with other genotypes. Nonetheless, this data was not statistically significant. Other risk factors of transmission such as intravenous drug use, imprisonment, piercing, and risky sex life were not reported more often and the difference was not statistically significant. However, in the majority of patients, the information about possible modes of transmission stayed unknown.

HBeAg seroconversion is an essential step in the natural course of HBV infection, and it is considered a positive outcome. HBeAg seroconversion is associated with a decline in HBV DNA levels. Patients with delayed or absent HBeAg seroconversion may progress to severe fibrosis and they are in a higher chance of developing hepatocellular carcinoma (18). Previous studies confirmed that the different HBV genotypes may influence the rate of HBeAg seroconversion. In our cohort, only eleven patients were HBeAg-positive. HBeAg positivity was significantly more frequent in patients infected with genotype D (n = 7; 9.2 %) than in those infected with genotype A (n = 2; 1.9 %), p = 0.037. Patients with HBV genotype D infection have a lower likelihood of spontaneous HBeAg seroconversion. Earlier HBeAg seroconversion is usually typical for those infected with HBV genotype A (12). These data also agree with our findings, since HBeAg-positive patients were mainly infected with HBV genotype D, which was statistically significant. Nonetheless, Sanchez-Tapiaz in a retrospective study of Spanish patients with chronic hepatitis B did not confirm any difference in the probability of HBeAg seroconversion between HBV genotypes A and D. Although, in HBV genotype A patients, there was a higher rate of remission after HBeAg seroconversion (55 % vs 32 %, p < 0.01) (19).

Patients who experienced delayed HBeAg seroconversion may have longer periods of high HBV DNA replication and a longer phase of hepatic inflammation with the consequent liver complication (20, 21). Patients with the infection HBV genotype D have more active liver disease and advanced liver fibrosis compared to those with genotype A infection (16). Our analysis did not confirm a statistically significant difference in the level of fibrosis and infection by different HBV genotypes, although, in the group of treated patients, more advanced fibrosis was observed in patients infected with HBV genotype D.

qHBsAg measurement may predict response to treatment and disease progression. Serum HBsAg levels show to correlate with other markers of HBV infection, including HBV genotypes (22). qHBsAg levels are linked with the progression of liver disease in HBeAg-negative genotypes B and C patients, but it is not clear whether this is consistent in all HBV genotypes (23). In our analysis untreated patients infected with HBV genotype A had higher levels of qHBsAg compared to patients infected with HBV genotype D (8 603 IU/mL vs 2 179 IU/mL). This difference was statistically significant (p < 0.0001). Vergori et al. in their study confirmed lower levels of qHBsAg in untreated patients with HBV genotype D vs A+E (p = 0.01) (24). The role of qHBsAg during antiviral therapy is still debatable. In our group of treated patients, we observed lower levels of qHBsAg in those infected with HBV genotype A vs D (but it was not statistically significant. Similar results were confirmed in a prospective study of 123 patients treated with entecavir, where different kinetics in HBsAg decline was shown. qHBsAg decline was significantly distinct in HBV genotype A vs D (p = 0.012) (25). In a large North American HBV epidemiological study qHBsAg significantly correlated with HBV DNA in treatment-naïve patients and with HBV genotype B, but not with liver fibrosis (26). Another study evaluated the correlation between HBV DNA and HBsAg level according to HBV genotype in 80 patients. They observed that qHBsAg level tended to correlate with HBV DNA level for genotype A (p = 0.02). Such correlation was not statistically significant for HBV genotype D (12, 27). More studies are needed to confirm the relationship between HBV genotypes and qHBsAg levels.

Conclusion

In conclusion, the genotypes of the hepatitis B virus play a significant role in the pathogenesis, clinical course, and response to treatment of the disease. We confirmed that the geographical distribution of HBV genotypes is not random and in the EU region, the most prevalent are HBV genotypes A and D. The optimal therapeutic goal of HBV infection is complete clearance of HBV. For now, we can only achieve a "functional cure", which is HBsAg loss or seroconversion. Unfortunately, the patient is still at risk of progression of the disease and developing hepatocellular carcinoma. Accurate identification of prognostic factors is, therefore, crucial for guiding clinical management and clinical strategies. The important prognostic factor is qHBsAg. In our study we confirmed, patients infected with HBV genotype A have higher levels of qHBsAg. Further research is needed to elucidate the mechanism underlying the differences between HBV genotypes and their clinical implication. We should consider testing patients with chronic hepatitis B for HBV genotypes routinely because the future of chronic hepatitis B treatment is towards its individualization. It will be important to know all prognostic markers that can play an important role in deciding on a therapeutic approach.

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