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Frequency of drug resistance and immune escape mutations in the hepatitis B virus genome detected in pregnant women in the Republic of Guinea

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The aim of the work is to assess the prevalence of hepatitis B virus drug resistance mutations and immune escape mutations in pregnant women in the Republic of Guinea.
Materials and methods. Blood plasma samples obtained from 480 pregnant women from different regions of the Republic of Guinea with laboratory-confirmed viral hepatitis B were studied. Nucleotide sequences for genotype identification and mutation detection were obtained using nested-PCR followed by Sanger sequencing, based on overlapping pairs of primers spanning the complete genome of the virus.
Results and discussion. In the examined group, the viral genotype E was the most prevalent (92.92%) compared with subgenotypes A1 (1.67%), A3 (1.46%), D1 (0.63%), D2 (1.04%) and D3 (2.29%). Among the examined HBV-infected pregnant women, 188 (39.17%) had undetectable HBsAg. Drug resistance mutations were detected in 33 individuals, which amounted to 6.88%. The following mutations were found: S78T (27.27%), L80I (24.24%), S202I (15.15%), M204I/V (42.42%). The presence of polymorphic variants not described as drug resistant has also been shown in positions associated with the development of drug resistance to tenofovir, lamivudine, telbivudine and entecavir (L80F, S202I, M204R). When analyzing the MHR and the region of *a* determinant, mutations were detected in 318 (66.25%) of pregnant women. In 172 of them, which amounted to 54.09%, multiple mutations were found. The amino acid substitutions in 13 positions associated with HBsAg-negative hepatitis B and/or potentially affecting HBsAg antigenicity were identified.
Conclusion. The high prevalence of immune escape and drug resistance mutations potentially associated with false-negative result of HBsAg screening, prophylaxis failure, and virological failure of therapy that has been identified among treatment naive pregnant women imposes a serious problem.

Keywords: *viral hepatitis B; occult hepatitis B infection; hepatitis B virus; drug resistance mutations; escape mutations; clinically significant mutations; laboratory diagnostics; pregnant women; Republic of Guinea*

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ОРИГИНАЛЬНОЕ ИССЛЕДОВАНИЕ

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Частота встречаемости мутаций лекарственной устойчивости и ускользания от иммунного ответа в геноме вируса гепатита В, выявленного у беременных в Гвинейской Республике

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Введение. Несмотря на все усилия по ограничению передачи вируса гепатита В (ВГВ) от матери ребёнку, понимание течения хронического гепатита В (ХГВ) у беременных женщин всё ещё ограниченно. Одним из регионов с крайне высокой распространённостью ХГВ является Африка: здесь суммарное количество больных составляет приблизительно 75 млн человек. Кроме того, серьёзным фактором, способным повлиять как на лечение, так и на вакцинную профилактику, могут являться мутации вируса. Таким образом, изучение генетической гетерогенности ВГВ является значимым.
Цель работы – оценить распространённость мутаций лекарственной устойчивости и мутаций ускользания от иммунного ответа ВГВ у беременных женщин в Гвинейской Республике.
Материалы и методы. Исследованы образцы плазмы крови, полученные от 480 беременных женщин из разных регионов Гвинейской Республики с лабораторно подтверждённым ВГВ. Нуклеотидные последовательности для определения генотипов и выявления мутаций получали с использованием nested-ПЦР с последующим секвенированием по Сэнгеру на базе перекрывающихся пар праймеров, совместно фланкирующих полный геном вируса.
Результаты и обсуждение. В обследованной группе чаще всего обнаруживали вирус генотипа E (92,92%) по сравнению с субгенотипами A1 (1,67%), A3 (1,46%), D1 (0,63%), D2 (1,04%) и D3 (2,29%). Среди обследованных ВГВ-инфицированных беременных было выявлено 188 человек (39,17%) с неопределяемым HBsAg. Мутации лекарственной устойчивости вируса были выявлены у 33 человек, что составило 6,88%. Обнаружены следующие мутации: S78T (27,27%), L80I (24,24%), S202I (15,15%), M204I/V (42,42%). Показано также наличие полиморфных вариантов, не описанных как фармакорезистентные, в положениях, связанных с развитием лекарственной устойчивости к тенофовиру, ламивудину, телбивудину и энтекавиру (L80F, S202I, M204R). При анализе MHR и региона детерминанты α мутации выявлены у 318 (66,25%) беременных. Из них у 172 человек, что составило 54,09%, обнаружены множественные мутации. Определено

наличие замен в 13 позициях, ассоциированных с HBsAg-негативным ХГВ и (или) потенциально влияющих на антигенность HBsAg.
Заключение. Выявленная среди терапевтически наивных беременных женщин широкая распространённость мутаций иммунного бегства и лекарственной устойчивости, способных приводить к ложноотрицательным результатам скрининга на HBsAg, безуспешной профилактике и вирусологической неэффективности терапии ВГВ-инфекции, представляет собой серьёзную проблему.

Ключевые слова: *вирусный гепатит В; скрытый гепатит В; вирус гепатита В; мутации лекарственной устойчивости; мутации вакцинного избегания; клинически значимые мутации; лабораторная диагностика; беременные; Гвинейская Республика*

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Introduction

The hepatitis B virus (HBV) is one of the most common hepatotropic viruses and can cause both acute and chronic liver disease. Currently, more than 360 million people have been diagnosed with chronic hepatitis B (CHB) [1]. Furthermore, 1.5 million new infections and 887,000 deaths caused by this chronic infection are reported annually [2]. The hepatitis B surface antigen (HBsAg) is the main marker for the laboratory diagnosis of infection; the frequency of its occurrence in population depends on the geographic region [3].

Africa is a region having an extremely high CHB prevalence: The HBsAg prevalence exceeds 8% and can be as high as 25%; the total number of people living with chronic HBV is approximately 75 million individuals and 25% of them are estimated to die from liver diseases [4]. In sub-Saharan Africa, including its western part, the main routes of transmission are vertical – mother-to-child transmission during birth – and horizontal – in early childhood through close household contacts with infected parents, siblings, or other relatives [2].

Perinatal and early childhood HBV infection not only increases the risk of developing chronic disease, but also is strongly predictive of poor long-term outcomes in patients, such as liver cirrhosis and hepatocellular carcinoma [5]. Screening for HBV in all pregnant women is of critical importance. Approximately 75 million women of reproductive age are chronically infected by HBV, accounting for nearly 25.3%. Without prophylaxis regimens, the rate of mother-to-child HBV transmission is 31.3% [6]. A strong focus on infected women and preventive treatment of women with a high viral load can be an efficient way to reduce the risk of infection transmission to a child [7]. Considering the goal set by the World Health Organization (WHO) calling for elimination viral hepatitis as a major public health threat by 2030, the reduction of mother-to-child virus transmission through universal immunization of newborns and administration of HBV immunoglobulin to infants born to infected women is among HBV prevention priorities [8]. Current therapies can rarely cure CHB due to the refractory nature of viral replication via the covalently closed circular HBV DNA, which resides in the nucleus of infected cells as an episomal plasmid and is responsible for continuous renewal of the viral pool in the body [9]. In the meantime, the treatment-induced reduction in the viral load

in pregnant women can significantly reduce the risk of prenatal infection of the child [10]. Despite all the efforts aimed at reduction of HBV mother-to-child transmission, the knowledge of CHB in pregnant women is still limited. Moreover, virus mutations can be a serious factor that can affect both treatment and preventive vaccination. Therefore, exploration of HBV genetic heterogeneity is a significant task of scientific research.

HBV is characterized by a high degree of genetic heterogeneity and is currently classified into ten genotypes (A–I), which differ in more than 8% of their nucleotide sequences. Genotypes A, B, C, D, F and H are further subdivided into subgenotypes having a divergence from 4% to 7.5% in nucleotide sequences [11]. In most of the geographic regions, with rare exceptions, 1 or 2 major and several minor genotypes, including the genotypes imported from other regions, prevail [12]. Genotypes A, D and E are the most common genotypes found in Africa. HBV genotype A is distributed worldwide and prevails in countries of South, Central and East Africa, while genotype E is dominant in the Western and Central part of the continent. At the same time, the prevalence of these genotypes can vary significantly even within the same country. The high degree of HBV genetic variability enables the virus to respond to endogenous and exogenous selective pressures by further modifications in its genome structure. During long-lasting infection and under various selective effects, some variants, especially in the *S* gene, can evolve, thus helping the virus escape therapeutic, preventive, and diagnostic measures [13].

The HBV envelope gene has three open reading frames – PreS1, PreS2 and S, which encode three proteins – small, middle, and large surface proteins translated from different messenger RNAs. The large HBV *S* gene encodes the preS1 protein (108 aa), the preS2 protein (55 aa) and the small S protein, or HBsAg (226 aa). Both before and after antiviral therapy, mutations in the S region (the region of the small HBV surface antigen) mainly occur in the N-terminal region (1–99 aa) and in the major hydrophilic region (MHR) (100–169 aa), but not in the C-terminal region (170–226 aa). In its turn, MHR harboring a relatively large number of amino acid substitutions contains the α-determinant region (124–147 aa) that owes its antigenicity to its tertiary structure. Thus, the effectiveness of vaccination and therapy can be reduced due to clinically significant mutations: immune escape mutations (EMs), drug resistance (DR) mutations, and mutations associated with disease progression [14]. Moreover, due to the overlapping of *S* and reverse transcriptase (RT) genes, DR mutations in the *RT* gene can induce EMs in MHR, and vice versa [15].

The Republic of Guinea located in West Africa is a region with a high prevalence of many viral infectious diseases, including those caused by hepatotropic viruses. This emphasizes the importance of epidemiological assessment of this territory and clear understanding of the situation for HBV [16]. At the same time, in Guinea, vaccination against HBV has been included in the national immunization schedule since 2006, but it is not mandatory for newborns. The recommended schedule includes two doses of the vaccine administered at 2 and 4 months of age, which are followed by revaccination at 11 months. Previously unvaccinated adolescents 11 through 15 years of age are administered a three-dose schedule or an alternative two-dose schedule completed at least 6 months apart. In the meantime, the Guinean Ministry of Health reported the vaccination coverage of only 47% of the population [17].

In 2021, Guinea developed new standards and procedures for the expanded vaccination program designed to start HB vaccination at birth; however, the offered schedule recommends several vaccination protocols – at birth and a three-dose series at 6, 10 and 14 weeks of age. It should be noted that medical supplies, including vaccines, are significantly limited in the Republic of Guinea not only because of the high costs of imported medical products, but also due to the lack of an established system of storage and transportation of cold chain products. Another problem is the medical mistrust among the population, including mothers' refusal to have their children vaccinated. There is no mandatory screening of the population, including pregnant women, for HBsAg in Guinea. HBV studies are limited in number and in the range of diagnostic methods applied. For example, there are only few publications addressing molecular and genetic characteristics of the virus [18]. We have previously reported escape mutations detected in pregnant women in Conakry; with such mutations, the virus can escape the HBsAg diagnostic test, causing its failure [19]. However, such tests have not been performed for pregnant women in Guinea.

The **purpose** of our study is to assess the prevalence of HBV-associated drug-resistance and escape mutations in pregnant women in the Republic of Guinea.

Materials and methods

The study was performed using plasma samples collected from 1,810 pregnant women from different regions of the Republic of Guinea. The age of the examined patients ranged from 13 to 55 years; the average age was 25.8 years. 402 women (22.21%) live in rural areas, and 1,408 (77.79%) live in urban areas. None of the women had previously been diagnosed with viral HBV; all the examined were screened for HBV markers during the study for the first time.

The laboratory tests were performed at the Russian–Guinean Research Center for the Epidemiology and Prevention of Infectious Diseases at the Research Institute of Applied Biology of Guinea (IRBAG) in the Kindia prefecture. This stage of study was approved by the Ethics Committee of Guinea (Minutes No. 129/CNERS/16 of August 31, 2015). All the examined individuals gave their written informed consent for participation in the study.

The HBV serological markers (HBsAg, anti-HBs IgG and anti-HBc IgG) were detected by the enzyme immunoassay using diagnostic kits manufactured by Diagnostic Systems RPC and Vector-Best JSC, following the previously described procedure [19]. The sensitivity of the test systems intended for HBsAg detection and used in this study was 0.01 IU/ml.

For detection of HBV DNA, we used the real-time polymerase chain reaction (PCR) with fluorescent hybridization probes and the technique developed at the St. Petersburg Pasteur Research Institute of Epidemiology and Microbiology; the technique is designed to detect HBV DNA in biological materials at low viral loads, including HBsAg-negative samples, providing the sensitivity of 10 IU/ml for extraction from 100 µl of plasma [16]. Nucleotide sequences for identification of genotypes and detection of mutations were obtained using nested PCR followed by Sanger sequencing and overlapping primer pairs flanking the entire virus genome, as it was described earlier [20]. The serotypes of the identified isolates, which characterized their antigenic specificity, were identified using the analysis of the nucleotide sequence of the conserved region of HBsAg α-determinant.

The statistical analysis of the data was performed using the Microsoft Excel, Prizm 5.0 software. The Clopper–Pearson (exact) interval was used to estimate statistical dispersion. The results are presented using a 95% confidence interval (CI). To assess the significance of differences in quantitative variables obtained from pairwise comparisons, we used, depending on the characteristics of the samples, Fisher's exact test or the χ^2 test with the Yates correction. The threshold for statistical significance was set at the probability value $p < 0.05$.

Results and discussion

The overall prevalence of HBV serological markers in the entire group was 56.63% (95% CI, 54.31–58.93%). The results of the analysis of prevalence of the studied HBV markers in the group are summarized in **Table 1**.

It should be noted that although anti-HBs IgG antibodies were detected in 37.13% of pregnant women, only one woman informed that she had been vaccinated against HBV; in all the other cases, most likely, antibodies were of natural origin through exposure to the virus.

During the assessment of the prevalence of the HBV molecular and biological marker, the viral DNA was detected in 480 women who accounted for 26.52% of all cases (95% CI, 24.5–28.62%).

The prevalence of HBV DNA in urban and rural areas was 26.92% (95% CI, 24.62–29.32%) and 25.12% (95% CI, 20.96–29.66%), respectively. Thus, no significant differences were found.

While the number of teen pregnancies is gradually decreasing in the world, the number of actual pregnancies among adolescent girls in Africa shows no decline. Although the legal age is set at 18 in the Republic of Guinea, the local cultural traditions are still respected, and a girl is considered ready for marriage and childbearing once she had her menarche. The age distribution among pregnant women in the examined group is shown in **Fig. 1**.

Given that WHO defines pregnancies that occur before and including the age of 19 as adolescent pregnancies increasing the risk of systemic infections and development of such gynecological diseases as puerperal endometritis and eclampsia, the group was broken down by age to identify the HBV DNA prevalence patterns in different age categories including groups ages 13 to 17 years (142 individuals, 7.85% of the total group), 13 to 19 years (382 individuals, 21.1% of the total group), 18 to 35 years (1,496 individuals, 82.65% of the total group) and over 35 years (172 individuals, 9.5% of the total group). The results are summarized in **Table 2**.

Significant differences were found in the HBV DNA prevalence between the groups aged 13–19 and 18–35 years: $\chi^2 = 8.346$ at $p = 0.0039$, df = 1, RR = 1.295 at 95% CI, 1.096–1.531%. However, no differences between groups aged 13–17 and 18–35 years were found: $p = 0.1297$.

The phylogenetic analysis of 480 isolates showed that the HBV genotype E had the highest detection frequency in the examined group (92.92%; 95% CI, 90.24–95.05%); the detection frequency was much lower for genotype A (3.13%; 95% CI, 1.76–5.1%) and genotype D (3.96%; 95% CI, 2.4–6.11%). The more thorough typing demonstrated the dominance of HBV genotype E (92.92%; 95% CI, 90.24–95.05%) compared to subgenotypes A1 (1.67%; 95% CI, 0.72–3.26%), A3 (1.46%; 95% CI, 0.59–2.98%), D1 (0.63%; 95% CI, 0.13–1.82%), D2 (1.04%; 95% CI, 0.34–2.41%) and D3 (2.29%; 95% CI, 1.15–4.06%) (**Fig. 2**).

Among serotypes of the detected isolates, the highest detection frequency rate was demonstrated by serotype ayw4 (92.92%; 95% CI 90.24–95.05%), which was represented by all isolates of genotype E. Consequently, much lower detection frequency was demonstrated by serotypes ayw2 (2.5%; 95% CI 1.3–4.33%), ayw3 (1,25%; 95% CI 0.46–2.7%), ayw1 (1.46%; 95% CI 0.59–2.98%), adw2 (1.67%; 95% CI 0,72–3,26%) and adw3 (0,21%; 95% CI 0,01–1.16%) (**Fig. 3**). Two serotypes were identified for genotype A – ayw1 and adw2, while for genotype D, three serotypes were identified – ayw2, ayw3 and adw3.

Table 1. Prevalence of HBV serological markers (HBsAg, anti-HBcore IgG, anti-HBs IgG) in the examined group (*n* = 1810)
Таблица 1. Распространённость серологических маркеров ГВ (HBsAg, анти-HBcore IgG, анти-HBs IgG) в обследованной группе (*n* = 1810)

Detected serological markers Выявленные серологические маркеры	Pregnant women, *n* (%) Беременные женщины, *n* (%)	95% Confidence Intervals, % 95% доверительный интервал, %
HBsAg+	292 (16.13)	14.47–17.91
HBs IgG+	672 (37.13)	34.90–39.40
HBcore IgG+	885 (48.90)	46.57–51.23
Seronegative / Серонегативные	785 (43.37)	41.07–45.69

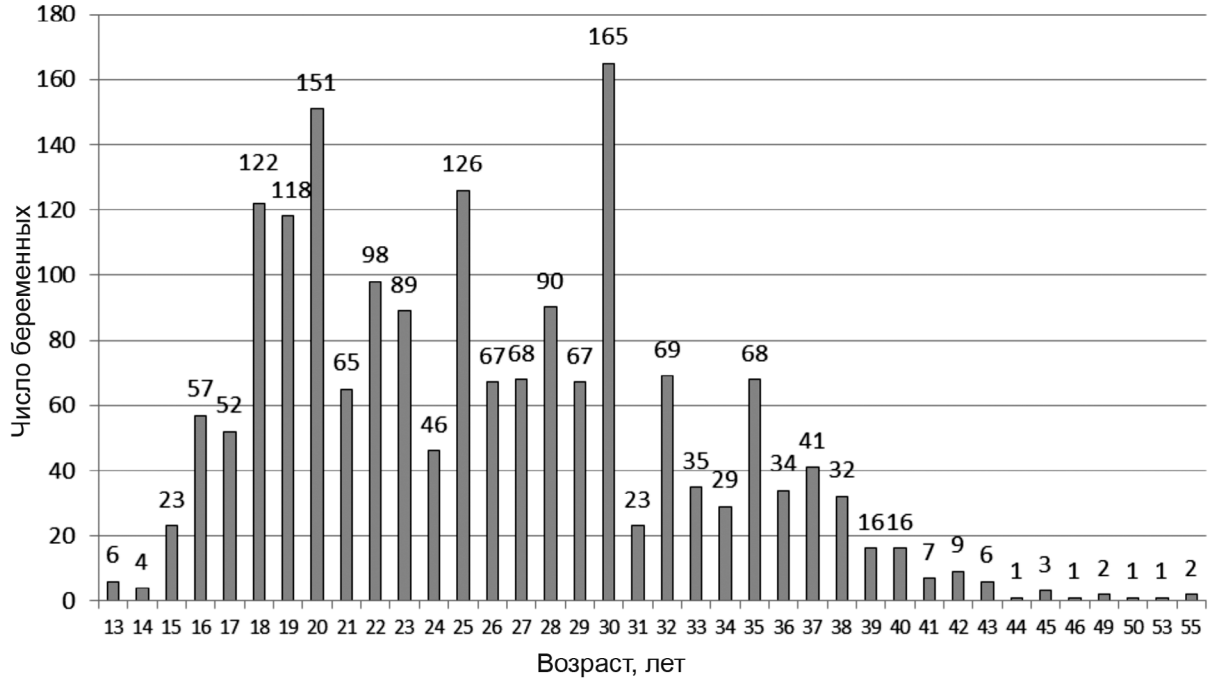

Fig. 1. Age distribution of pregnant women in the study group.
Рис. 1. Распределение по возрастам беременных женщин в обследуемой группе.

Table 2. Prevalence of HBV DNA in different age groups
Таблица 2. Распространённость ДНК ВГВ в разных возрастных группах

Age groups Возрастные группы	Pregnant women, *n* (%) Беременные женщины, *n* (%)	95% Confidence Intervals, % 95% доверительный интервал, %
13–17 years / лет	45 (31.69)	24.14–40.02
13–19 years / лет	126 (32.98)	28.29–37.95
18–35 years / лет	381 (25.47)	23.28–27.76
>35 years / лет	54 (31.4)	24.55–38.9

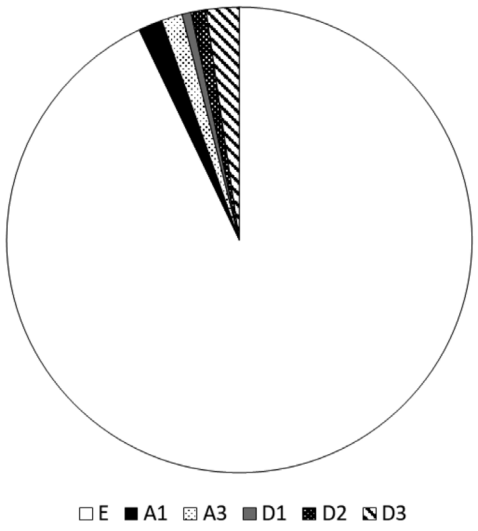

Fig. 2. Distribution of HBV subgenotypes in the examined group.
Рис. 2. Распределение субгенотипов вируса гепатита В в обследованной группе.

The distribution of HBV genotypes in the group shows consistency with the data on the virus variants circulating in the region. We had previously reported that in the Republic of Guinea, HBV genotype E was dominant among blood donors (85.53%) [21]. Genotype E was also the most prevalent genotype in the neighboring countries. For example, in Mali, the prevalence of this genotype is 91.1%, in Côte d'Ivoire – 87.4%, in Senegal and Burkina Faso – 75 and 72%, respectively [22]. Although HBV genotype E is widespread in sub-Saharan Africa, it is characterized by low genome variability, which may indicate its relatively short evolutionary pathway. Genotype E has an epidemiological relationship with the HBsAg-negative infection and is associated with development of DR mutations and vaccine escape mutations [22]. Among the examined HBV-infected pregnant women, 188 women (39.17%; 95% CI, 34.77–43.69%) had undetectable HBsAg, including 113 women under 30 years old, accounting for 60.11% (95% CI, 52.73–67.6%) of all pregnant women with occult hepatitis B infection (OBI). At the same time, HBsAg-negative and positive cases did not have any differences in the distribution of virus genotypes: $p = 0.9914$. The prevalence of HBV genotype E was 92.55% (95% CI, 87.82–95.87%) among women with OBI and 93.15% (95% CI, 89.62–95.77%) among HBsAg-positive pregnant women, thus being comparable with the total prevalence of this genotype in the examined group – 92.92%. In the meantime, in individuals with OBI, neither nucleotide nor amino acid sequences of the HBV Pre-S1/Pre-S2/S region did not demonstrate any signs of clustering and were distributed among the sequences identified in HBsAg-positive individuals. The phylogenetic tree including the studied isolates of HBV genotypes A and D is presented in **Fig. 4**.

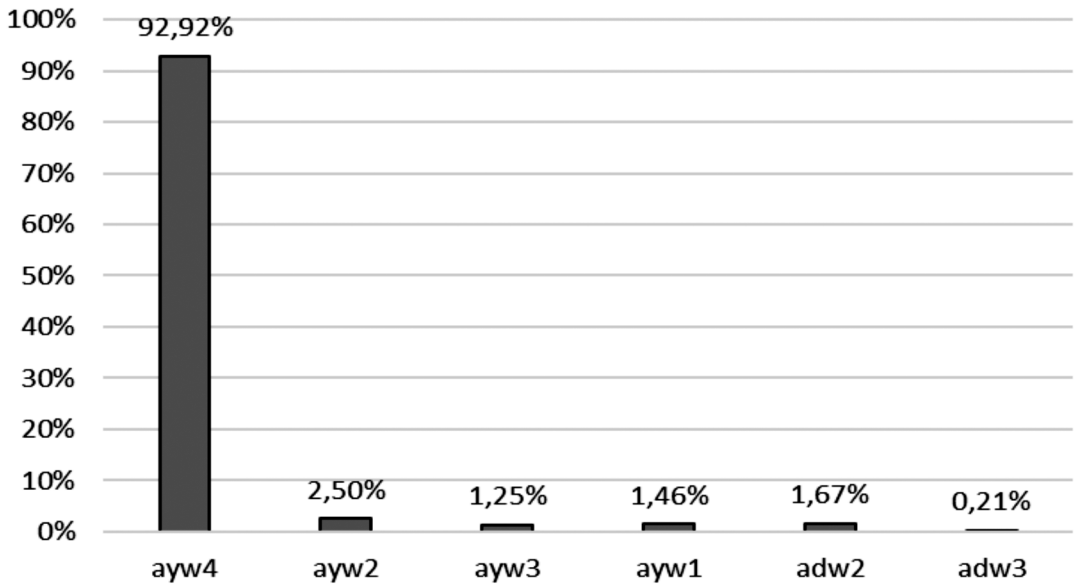

Fig. 3. Distribution of HBV serotypes in the examined group.
Рис. 3. Распределение серотипов вируса гепатита В в обследованной группе.

A fragment of the phylogenetic tree of HBV genotype E is shown in **Fig. 5**.

It can be assumed that the high prevalence of HBsAg-negative HBV in young women is associated with the specific patterns of infection transmission in African countries, following predominant infection in early childhood, including vertical transmission.

A significant number of mutations were detected in MHR and RT region. The genetic changes associated with amino acid substitutions in the RT domain are classified as primary DR mutations and secondary compensatory mutations that restore the replication ability of the virus. In this study, the analysis of the RT region of HBV genome revealed DR mutations in 33 individuals accounting for 6.88% (95% CI 4.78–9.52%) of cases. During the analysis of 33 isolates obtained from individuals with drug-resistant variants of the virus, DR mutations were detected in the following proportions: S78T (27.27%; 95% CI 13.3–45.52%), L80I (24.24%; 95% CI 11.09–42.26%), S202I (15.15%; 95% CI 5.11–31.9%), M204I/V (42.42%; 95% CI 25.48–60.78%). The S78T + L204I combination was detected in one individual, while three individuals had the L80I + M204I/V combination.

Atripla is the most frequently used antiviral medication in the Republic of Guinea for HBV treatment; it includes efavirenz, emtricitabine and tenofovir. Another popular medication is lamivudine. Therefore, the detection of mutations conferring resistance to tenofovir and lamivudine should come as no surprise. However, tenofovir has a high genetic barrier to resistance and effectively suppresses HBV both in mono-infected individuals and in individuals with human immunodeficiency virus (HIV)/HBV coinfection [13]. In addition, all examined pregnant women denied that they had been given the above drugs or any other drug therapy. Assumedly, the mutations detected in therapeutically naive women were developed through the infection with the mutation-carrying virus transmitted from their partners having poor or moderate adherence to the prescribed treatment. Such genetic changes can also be caused by the natural polymorphism of the virus. This assumption is indirectly confirmed by the fact that mutations were detected only in patients with HBV genotype E as well as by the presence of polymorphic variants that are not described as drug resistant at the positions associated with development of DR to tenofovir, lamivudine, telbivudine and entecavir (L80F, S202I, M204R). In some regions of Africa, the above DR mutations were detected in therapeutically naive individuals much more frequently than in European countries or American countries [13]. The S78T mutation deserves special attention: In addition to the increased production of HBV transcripts and the sustained secretion of viral particles in the absence of detectable antigenic domains of S protein, this amino acid substitution may predispose patients to carcinogenic effects [23]. It should be noted that in our studies of viral hepatitis in the Republic of Guinea, such mutations had not been previously detected among HBV/HIV-coinfected individuals or among relatively healthy people and blood donors, though amino acid substitutions in DR-related positions had been detected. It should also be noted that although for treatment-naive HIV-infected individuals, the established threshold for the prevalence of primary DR mutations is > 5%, above which HIV DR mutations pose a challenge for successful treatment at the population level, requiring the country-wide analysis of primary resistance in all infected individuals and the subsequently selected treatment regimen, there are no recommendations for establishing any threshold for the prevalence of such mutations in HBV-infected indi-

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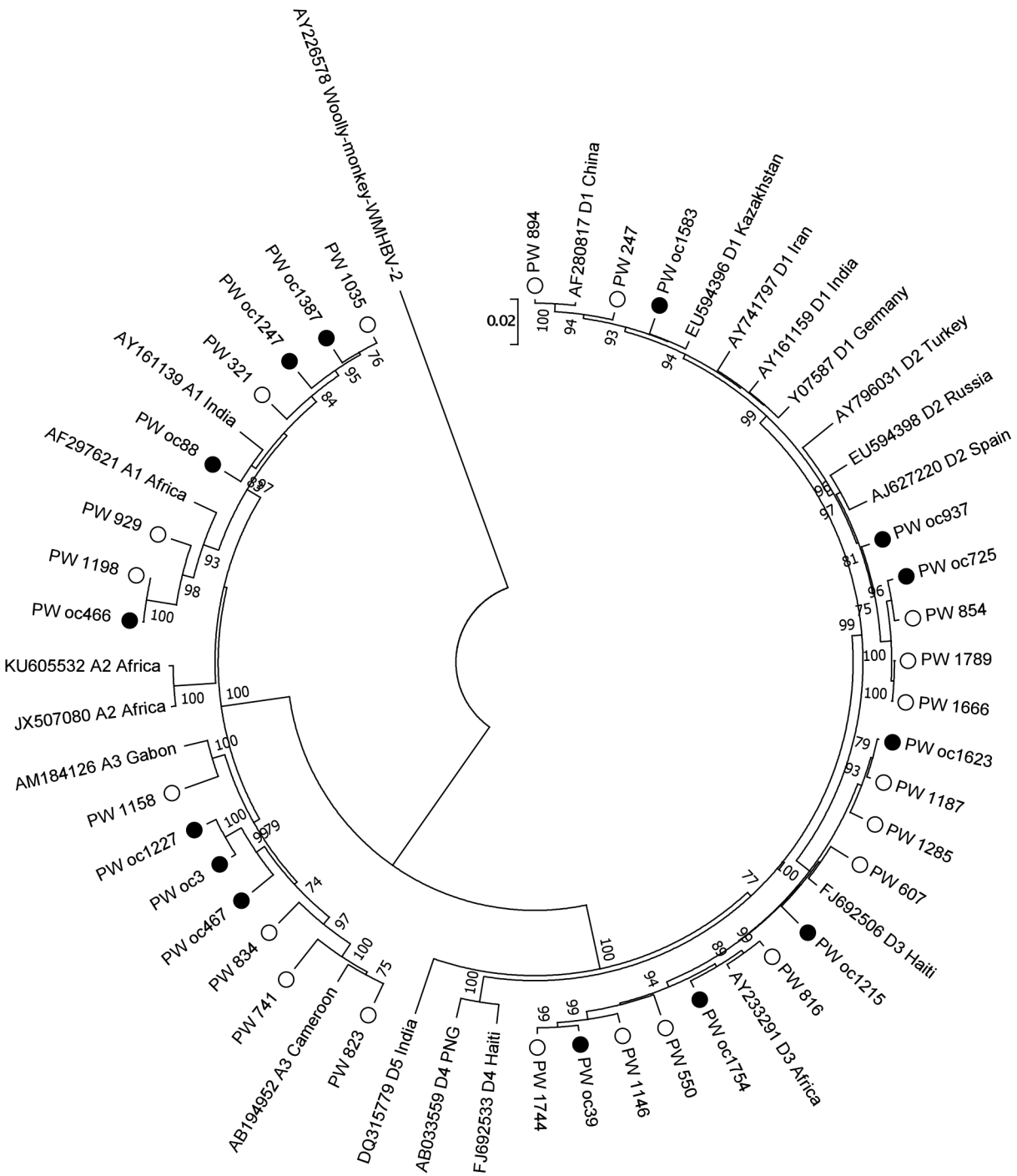

Fig. 4. Phylogenetic tree for HBV complete genome nucleotide sequences of genotypes A and D isolated from pregnant women living in the Republic of Guinea in comparison with the reference sequences presented in the GenBank database. Reference sequences are designated with GenBank accession numbers and indicated genotype and the region of the sample origin. The Woolly Monkey HBV nucleotide sequence AY226578 was used as the outgroup. The sequences from this study are indicated by white circles (HBsAg+) and black circles (HBsAg–). Bootstrap values ≥ 70.

Рис. 4. Филогенетический анализ нуклеотидных последовательностей полных геномов вируса гепатита В генотипов A и D, выделенных от беременных, проживающих в Гвинейской Республике, в сравнении с представленными в международной базе данных GenBank референсными последовательностями. Референсные последовательности обозначены кодами GenBank с указанием генотипа и региона происхождения образца. Как внешняя группа использована нуклеотидная последовательность вируса гепатита В шерстистой обезьяны AY226578. Исследованные в настоящей работе образцы обозначены белыми (HBsAg+) и чёрными кружками (HBsAg–). Даны значения bootstrap ≥ 70.

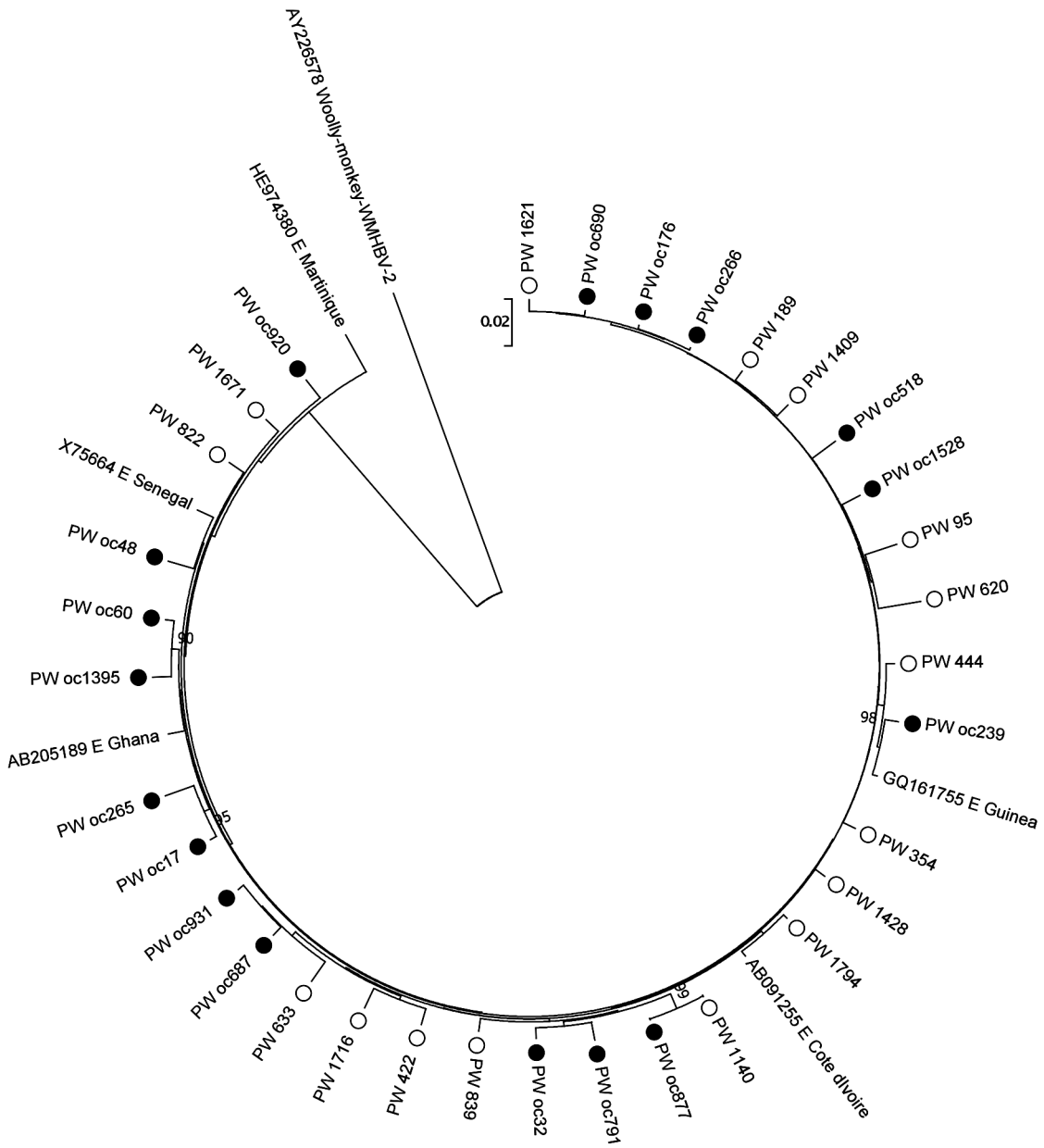

Fig. 5. Phylogenetic tree for some HBV complete genome nucleotide sequences of genotype E isolated from pregnant women living in the Republic of Guinea in comparison with the reference sequences presented in the GenBank database. Reference sequences are designated with GenBank accession numbers and indicated genotype and the region of the sample origin. The Woolly Monkey HBV nucleotide sequence AY226578 was used as the outgroup. The samples studied in this work are indicated by white circles (HBsAg+) and black circles (HBsAg–). Bootstrap values ≥ 70.

Рис. 5. Филогенетический анализ некоторых нуклеотидных последовательностей полных геномов вируса гепатита В генотипа Е, выделенных от беременных, проживающих в Гвинейской Республике, в сравнении с представленными в международной базе данных GenBank референсными последовательностями. Референсные последовательности обозначены кодами GenBank с указанием генотипа и региона происхождения образца. Как внешняя группа использована нуклеотидная последовательность вируса гепатита В шерстистой обезьяны AY226578. Исследованные в настоящей работе образцы обозначены белыми (HBsAg+) и чёрными кружками (HBsAg–). Даны значения bootstrap ≥ 70.

viduals. Clearly, the specification of the similar threshold values and recommendations for HBV would contribute to the assessment of the epidemiological situation in regions and to the unification of treatment strategies.

DR mutations can also cause genetic changes in MHR due to the overlap between the genes encoding RT and HBsAg. So far, more than 30 HBsAg mutations have been identified. These mutations help the virus escape immune responses: They allow the virus to evade neutralizing antibodies, promote persistent HBV infection and virus adaptability. Immune-associated EMs can also interfere with HBsAg recognition by vaccine-induced antibodies, posing a potential threat to the global vaccination program [24].

During the analysis of MHR and the α-determinant region, which is a cluster of major B-cell epitopes located between 124 and 147 (or 149) aa positions, mutations were detected in 318 of 480 pregnant women (66.25%; 95% CI, 61.83–70.47%). Multiple genetic changes were detected in 172 of 318 individuals with detectable mutations in the α-determinant, accounting for 54.09% (95% CI, 48.44–59.66%). Substitutions associated with OBI and (or) potentially affecting HBsAg antigenicity were detected at 15 positions. The clinically significant mutations prevalent in the examined group of HBV-infected pregnant women are presented in **Table 3**.

Among the DR mutations in the RT region, which were detected during this study, only two mutations result in significant amino acid substitutions in the S region of the virus genome. For example, the rtS78T/sC69*, mutation creates a premature stop codon and thereby deletes almost the entire small HBV surface protein [23]. In its turn, the rtM204I/V polymorphism results in two mutations in the *S* gene: sI195M associated with reduced antibody reactivity to epitopes in the first or second loop, or in both loops of the protein [25], and sW196* resulting in the C-terminal truncation of the protein, promoting cell transformation and associated with a high oncogenic potential due to the altered host gene expression [26, 27].

As has been found previously, the average mutation frequency in genes encoding HBsAg is 11% in North American population and reaches 47% in CHB in South Korea, while the mutation frequency in MHR ranges from 57.5% for HBV genotype A to 100 % for HBV genotype D, amounting to 59.9% for HBV genotype E [28], thus demonstrating the consistency with the large number of substitutions detected in the examined group during our study.

Among the detected mutations, two mutations (Y100C, M103I) are associated with the development of HBsAg-negative CHB [29]. The I110L mutation is associated with immunoglobulin resistance. Ten mutations (T127P, Q129H/R, M133I, Y134H, C137Y, S140L, K141E, D144E, G145R, Y147C) were detected in the determinant region. Note that the most prevalent polymorphic variants associated with immune escape and detected in HBV isolates in genotype E are T116N, P120L/S, Q129H/R, M133I, D144E and G145I [30]; however, in the examined group, the last two mutations were detected quite rarely. In MHR, which contains two loops linked

Table 3. The most prevalent clinically significant mutations identified in the Pre-S1/Pre-S2/S-region sequences from the examined group (*n* = 480)

Таблица 3. Наиболее распространённые клинически значимые мутации, выявленные в регионе Pre-S1/Pre-S2/S в обследуемой группе (*n* = 480)

HBV genome region Область генома ВГВ	Mutation Мутация	Frequency of occurrence in the group Частота встречаемости в группе	Genotype Генотип
RT	S78T	9 (1.88%; 95% CI, 0.86–3.53%)	E
RT	L80I	8 (1.67%; 95% CI, 0.72–3.26%)	E
RT	S202I	5 (1.04%; 95% CI, 0.34–2.41%)	E
RT	M204I/V	14 (2.92%; 95% CI, 1.60–4.85%)	E
RT	L80F	17 (3.54%; 95% CI, 2.08–5.61%)	E
RT	S202R	19 (3.96%; 95% CI, 2.40–6.11%)	E
RT	M204R	53 (11.04%; 95% CI, 8.38–14.19%)	E
MHR	Y100C	21 (4.38%; 95% CI, 2.73–6.61%)	A, E
MHR	M103I	13 (2.71%; 95% CI, 1.45–4.59%)	E
MHR	I110L	65 (13.54%; 95% CI, 10.61–16.93%)	A, D, E
MHR	T116N	58 (12.08%; 95% CI, 9.30–15.34%)	E
MHR	P120L/S	94 (19.58%; 95% CI, 16.13–23.42%)	E
MHR	T127P	27 (5.63%; 95% CI, 3.74–8.08%)	D, E
MHR	Q129H/R	34 (7.08%; 95% CI, 4.95–9.76%)	D, E
MHR	M133I	110 (22.92%; 95% CI, 19.23–26.94%)	A, D, E
MHR	Y134H	31 (6.46%; 95% CI, 4.43–9.04%)	E
MHR	C137Y	46 (9.58%; 95% CI, 7.10–12.58%)	E
MHR	S140L	19 (3.96%; 95% CI, 2.40–6.11%)	E
MHR	K141E	39 (8.13%; 95% CI, 5.84–10.94%)	E
MHR	D144E	15 (3.13%; 95% CI, 1.76–5.1%)	E
MHR	G145R	28 (5.83%; 95% CI, 3.91–8.32%)	E
MHR	Y147C	23 (4.79%; 95% CI, 3.06–7.1%)	E

by disulfide bridges between cys124 and cys137 as well as cys139 and cys147, mutations destroy these bridges. The *in-silico* structure prediction studies suggested that the three-dimensional conformation of the extravirion loop of isolates having amino acid substitutions between 133 and 144 aa is different from that of the wild-type genotype E virus [31]. Such substitutions can lead to the production of a surface antigen, which is different from the typical one and which, consequently, is not detectable by tests [32, 33]. The G145R mutation can also impair HBsAg secretion and result in reinfection in liver tissue despite the presence of protective anti-HBs titers [34]. The Y147C mutation promotes replication during the viral DNA synthesis and, given the significance of the Cys-Cys disulfide bond in maintaining the conformation required for HBsAg antigenicity, results in breakthrough infection in vaccinated patients [35]. A number of mutations potentially capable of affecting the detection of HBsAg were detected outside the determinant region – G159A, E164G, A166V, S167L, A168V [29].

The HBV genotype sequence used for vaccines may potentially have an influence on immunogenicity against non-vaccine genotypes, but there are limited data to support this assumption [36]. The occurrence of HBV infection, even though immunoprophylaxis is administered, is associated either with vertical transmission of the virus harboring EMs or with selection of *de novo* mutations, especially when vaccination is delayed. To prevent vertical transmission of the virus, WHO recommends that all infants, regardless of the infection status of the mother, receive the first dose of the HB vaccine as soon as possible after birth, preferably within 24 hours, followed by 2 or 3 doses of the vaccine at least 4 weeks apart to complete the vaccination series [1]. However, administration of the birth dose of HBV vaccine is still limited in many low-income countries. Among 47 countries in the African Region, only 13 countries had introduced monovalent hepatitis B vaccination for infants by 2020 [37]. In most African countries where HBV immunization programs are being introduced by the government, as in the Republic of Guinea, vaccination is delayed until 6 weeks of age [38]. In addition, such factors as the high prevalence of home births as well as parental refusal to have their newborns examined and (or) vaccinated posed a challenge for timely preventive measures [39, 40]. It can be assumed that such delays not only create the window for infection, but also enhance the likelihood of EM variant transmission and/or development of new escape mutations.

The emergence of highly prevalent EMs in the population poses a threat to immunocompromised individuals, as evidenced by the study conducted by Salpini et al., who demonstrated that in 75% of patients with immunosuppression and HBV reactivation, the virus carries at least one mutation in the Pre-S1/Pre-S2/S, primarily located in MHR [41].

Note that in most cases, immune escape mutations occur concurrently with significant amino acid substitutions in *Pol* and *Core* genes, which are associated with virus DR or an increased risk of development of liver cirrhosis and hepatocellular carcinoma. Therefore, the awareness of the functional impact of EMs on HBV replication is highly important, as their presence leads to an increase in the prevalence of undetectable viral variants carrying mutations that are significant for disease treatment and disease progression. Coexistent complex HBV mutants, such as immune escape and DR mutants, raise concerns as they can affect vaccinated individuals, and require revision of the antiviral treatment and infection prevention strategies.

Conclusion

While the information about genetic diversity of HBV and clinically significant mutations is of critical significance in patient management, it is limited in the Republic of Guinea. Considering the global significance of the disease caused by this pathogen and the multifaceted impact of HBsAg protein variations on diagnostic test results, the clinical course of the disease and the effectiveness of its treatment, the comprehensive knowledge of the diversity and frequency of mutations is of fundamental importance. The high prevalence of immune escape and DR mutations detected among therapeutically naive pregnant women raises concerns, as such mutations can create false-negative HBsAg test results, compromise prevention strategies and affect the virological effectiveness of HBV infection therapies. Timely detection of the pathogen, genotyping, and identification of clinically significant mutations are critically important for prevention of vertical infection, disease treatment, identification of channels of infection and development of the immunization program in the Republic of Guinea.

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